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Asymmetric Reduction of Ketones

by *Geotrichum candidum*

2000

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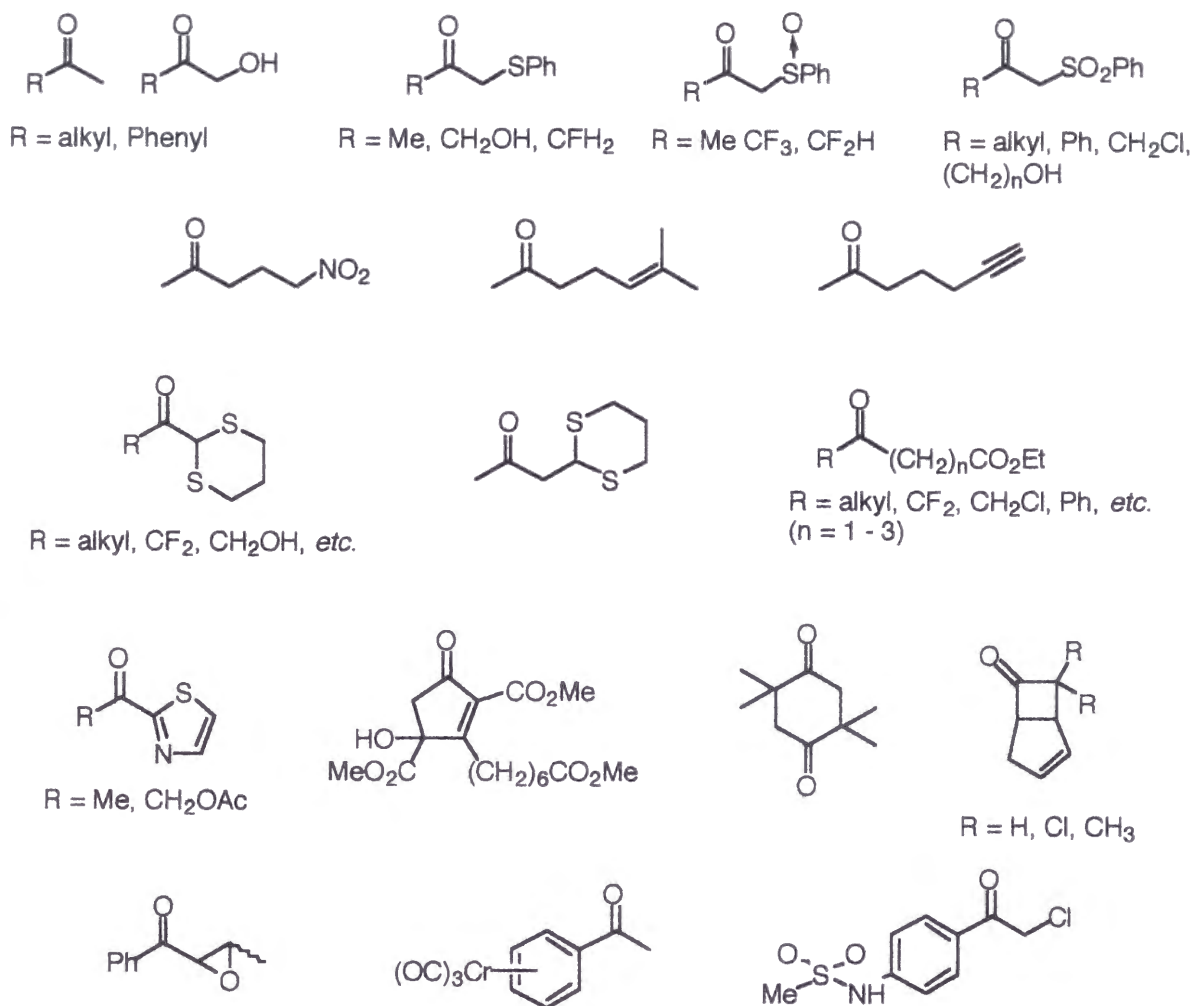
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I. Introduction

The synthesis of enantiomerically pure compounds is becoming increasingly important for research and development in chemistry and biochemistry.¹ Among chiral compounds, enantiomerically pure alcohols are particularly useful as building blocks for the synthesis of natural products, pharmaceuticals and agricultural chemicals since a variety of methods have been developed to convert alcohol functionality to other useful functional groups such as chloride,² amine,³ azide,⁴ fluoride,⁵ *etc.* One of the easiest methods for the preparation of optically active alcohols is the asymmetric reduction of ketones by either organometallic reagents with chiral ligands or biocatalysts such as alcohol dehydrogenases. The use of enzymatic reduction has attracted more and more attention due to the high stereoselectivity.⁶ Currently, alcohol dehydrogenases from various sources such as baker's yeast,⁷ Horse Liver (HLADH),⁸ *Thermoanaerobium brockii* (TBADH),⁹ *Thermoanaerobacter ethanolicus*,¹⁰ *Lactobacillus kefir*,¹¹ *Pseudomonas sp.*,¹² *Gluconobacter oxydans*,¹³ *Bacillus stearothermophilus*,¹⁴ *Geotrichum candidum*,¹⁵ *et al.* are used to reduce a full range of carbonyl compounds. Extensive research has been undertaken on the development of enzymatic systems for the asymmetric reduction of ketones and many methods to control the stereochemistry of the reduction have been reported.¹⁶

However, there is still a need to improve the enantioselectivity of biocatalytic reduction. Although the selectivities of previously reported reduction systems were relatively high (around 90 - 95% ee) to moderate, enantiomerically pure compounds (>99% ee) could not be obtained except for a few cases. For example, the selectivity of the reduction of ketones by permeabilized cells of *Gluconobacter oxydans* is around 95% for most of its substrates (2-pentanone, 2-hexanone, 2-octanone, 3-nonanone, cyclohexyl methyl ketone), but a selectivity of 99% ee is obtained only for the and methyl isopropyl ketone.¹³

Moreover, the substrate specificity of most of the systems is not wide enough to be beneficial for organic synthesis. Some reasons being, 1) the reactivity of HLADH is high for cyclic ketones but not for acyclic ketones^{8c} 2) the selectivity of TBADH is shown to be excellent for the reduction of only aliphatic ketones⁹ 3) when the substrate specificity with reduction by the whole cell, such as baker's yeast which is easy to manipulate and commercially available, is very wide, the selectivity is not always high.^{7b}



Some examples of keto compounds reduced by baker's yeast or by other microorganisms into the corresponding secondary alcohols with a high enantiomeric excess.^{6d}

Discovery of an easily available and handy biocatalyst with very high selectivity toward the reduction of any ketones, either aromatic or aliphatic, under mild reaction conditions is awaited. This thesis describes about an enzymatic reduction system by which both aromatic and aliphatic ketones can be reduced with excellent stereoselectivity, resulting in the synthesis of secondary alcohols with a high yield and >99% ee. It should be also emphasized that the biocatalyst for the system is easy to prepare and handle, the reaction proceeds under mild conditions since it is an enzymatic system, and the product isolation is also simple. Another advantage of this system is that it is environmentally friendly since harmful transition metals are not necessary.

A biocatalyst prepared from a dimorphic fungus, *Geotrichum candidum* IFO 4597, was employed for the reduction of ketones owing to its high reactivity with unnatural substrates and the

simplicity of its growth not requiring the assistance of a microbiologist. The acetone powder¹⁷ of *G. candidum* IFO 4597 (APG4), a microbial dried-cell dehydrated using acetone, was used as a catalyst for the asymmetric reduction of ketones for the first time and found that a number of ketones from aromatic ketones, β -keto esters, and simple aliphatic ketones are reduced to (*S*)-alcohol with >99% ee. The properties of APG4, such as coenzyme dependence, kind of alcohols to be adapted for coenzyme regeneration, kind of suitable buffer, optimum pH, optimum reaction temperature, stability, and preservability are examined, and then the scope and limitations of the system for substrate specificity are investigated.

The substrate specificity of the APG4 system has also been expanded particularly to trifluoromethyl ketones to prepare optically pure trifluoromethylalcohols due to their potential use¹⁸ as ferroelectric liquid crystals, drugs and tools for metabolic studies. Surprisingly, reduction of trifluoromethyl ketones by the APG4 system affords the corresponding alcohols of the opposite configuration of that of methyl ketones in excellent ee. The mechanism for the opposite stereochemistry was elucidated by enzymatic studies.

The by the APG4 under various conditions is described in Section II, reduction of various unfluorinated ketones in Section III, and reduction of trifluoromethyl ketones in Section IV.

II. Reduction of Acetophenone

Acetophenone were reduced with excellent selectivity to (*S*)-1-phenylethanol by using the acetone powder of *Geotrichum candidum*. This method is superior in reactivity and stereoselectivity to reduction by the whole-cell. The experimental conditions for the reduction system such as ratio of the biocatalyst to the substrate, kinds of coenzyme, alcohol for coenzyme regeneration, and buffer, pH, and reaction temperature were investigated and stability and preservability of the biocatalyst were examined.

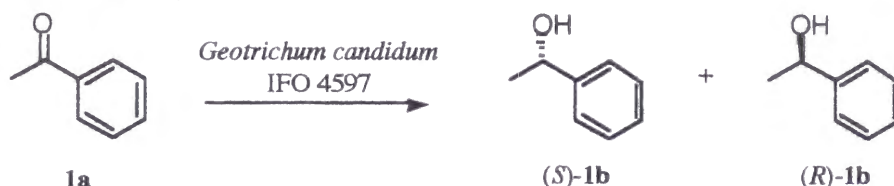
A. Preparations of the Enzyme (APG4).

The crude alcohol dehydrogenase, the acetone powder of *Geotrichum candidum* IFO 4597 (APG4), was prepared as usual¹⁷ by treating wet cells with acetone. The cultivation of *G. candidum* is very easy, requiring little technique, and the preparation of the acetone powder is also very simple and does not need any special equipment; mixing of the cold acetone to cells (18 g wet wt. of the whole cells cultured in a 1 L complex medium for 1 day) followed by filtration and drying of the precipitate yields as much as 3.8 g of the powder, APG4, within an hour. The simpleness of APG4 preparation is in striking contrast to the tediousness associated with isolation of an enzyme in a pure form.

B. Reaction Conditions for Asymmetric (1a).

The asymmetric (1a) by *G. candidum* was investigated, and the results are shown in Table 1. The (1a) catalyzed by the whole cell resulted in poor enantioselectivity (28 % ee(*R*)) thus it is necessary to improve the selectivity for the practical use of the microbe as a reagent for asymmetric reduction. The change in the form of the catalyst from wet whole-cell to dried powdered-cell (APG4) dramatically decreased the yield of the reduction of 1a, and no (*R*)-alcohol or (*S*)-alcohol was produced under the catalysis of APG4, which would indicate the loss of the necessary coenzyme(s) and/or coenzyme regeneration system(s) during the treatment of the cells with acetone. Addition of a coenzyme, NAD⁺, did not have a significant effect on the yield. Addition of 2-propanol resulted in only a small increase in the yield, but a significant improvement in the enantioselectivity was observed. Surprisingly, addition of both NAD⁺ and 2-propanol largely enhanced both chemical yield and enantiomeric excess (ee), which resulted in affording enantiomerically pure (*S*)-phenylethanol ((*S*)-1b) with 89% yield. Addition of NADH, NADP⁺ or NADPH instead of NAD⁺ and addition of cyclopentanol instead of 2-propanol also gave similar results, giving an enantiomerically pure alcohol in high yield.

Table 1. Reduction of **1a** by *G. candidum*



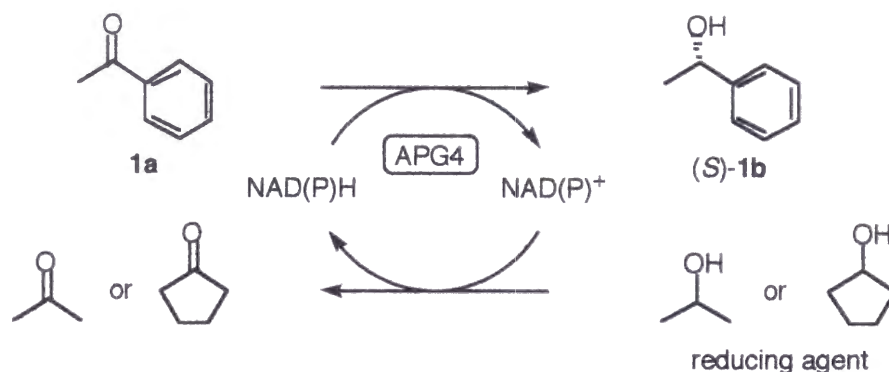
Catalyst	Coenzymed	Reducing Agent ^e	Yield(%) ^f	ee(%) ^f	Config. ^g
Whole cell ^b	none	none	52	28	<i>R</i>
APG4 ^c	none	none	0	-	-
APG4 ^c	NAD ⁺	none	1	71	<i>S</i>
APG4 ^c	NADP ⁺	none	0	-	-
APG4 ^c	none	2-propanol	8	98	<i>S</i>
APG4 ^c	NAD ⁺	2-propanol	89	>99	<i>S</i>
APG4 ^c	NADH	2-propanol	89	>99	<i>S</i>
APG4 ^c	NADP ⁺	2-propanol	88	>99	<i>S</i>
APG4 ^c	NADPH	2-propanol	83	>99	<i>S</i>
APG4 ^c	NAD ⁺	cyclopentanol	97	>99	<i>S</i>
APG4 ^c	NADP ⁺	cyclopentanol	86	>99	<i>S</i>

Reaction Conditions: 20 h at 30 °C at 130 rpm in water (3 mL) for whole-cell reaction or in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3 mL) for APG4 reactions. ^a 0.08 mmol. ^b 0.5 g wet weight. ^c 10 mg. ^d 0.007 mmol. ^e 1.31 mmol. ^f Determined by GC analyses. ^g Determined by comparison of the GC retention times with those of authentic samples.

The reaction mechanism is shown in Scheme 1. When acetophenone (**1a**) is reduced to (*S*)-1-phenylethanol ((*S*)-**1b**), the reduced form of the coenzyme (NAD(P)H) is oxidized to NAD(P)⁺, which is subjected to concomitant recycling to its reduced form by a reducing agent, such as 2-propanol or cyclopentanol. As the catalytic cycle is effective only for the reduction to the (*S*)-alcohol and does not involve any (*R*)-producing enzyme(s), the selectivity of the APG4 reduction system consisting of APG4, NAD(P)H, and a reducing agent is excellent, whereas the selectivity of the whole-cell reduction in which both of (*S*)-enzyme(s) and (*R*)-enzyme(s) are active to produce both enantiomers of the alcohol is low (28 % ee(*R*)).

The experimental conditions for the reduction system such as ratio of the biocatalyst to the substrate, kinds of coenzyme, alcohol for coenzyme regeneration, and buffer, pH, and reaction temperature are further investigated, and stability and preservability of the biocatalyst are also examined.

Scheme 1



Ratio of Biocatalyst (APG4) to Substrate (B/S).

The effect of the ratio of the biocatalyst (APG4) to the substrate (B/S) on the yield of (**1a**) was investigated, and it was found that only 0.4 of the B/S ratio was sufficient as shown in Figure 1. One of the troublesome problems usually encountered in biocatalytic processes in organic synthesis is the requirement of an excessively high weight of the biocatalyst compared to the substrate.^{6b,c,19} For example, in the baker's yeast (**1a**), a B/S ratio of 360 (1800 g of baker's yeast to reduce 5 g of **1a**) is necessary to obtain a 23% yield of (*S*)-1-phenylethanol ((*S*)-**1b**).^{7a} When the whole cell of *G. candidum* was used to reduce **1a**, a B/S ratio of 50 is necessary.^{15e} An advantage of the present system is that only a very small amount of the biocatalyst, a B/S ratio of 0.4, is sufficient to reduce **1a** with a 89% yield compared with a ratio for baker's yeast of 360, or for whole cell of 50.

The enantioselectivity of the reduction was not affected by the amount of the catalyst at all, and perfect selectivity (>99% ee) was obtained regardless of the B/S ratio.

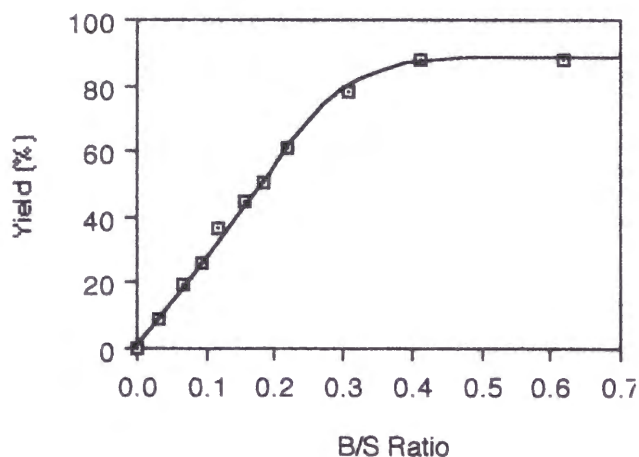


Figure 1. Effect of biocatalyst / substrate ratio (B/S Ratio) on the reduction of **1a**.

Reaction Conditions: 20 h at 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. Yields and ee were determined by GC analyses, and ee was >99 %(*S*) for any amount of APG4 employed.

Coenzymes.

Unlike biotransformations using the whole cell, a coenzyme must be replaced in enzymatic transformations. Using 2-propanol as a reducing agent, the effectiveness of several nicotinamide coenzymes for the (1a) was investigated. As shown in Table 1, NAD⁺, NADH, NADP⁺ and NADPH can be used to reduce acetophenone (1a) with high yield. The selectivity of the reduction was hardly affected by the kind of coenzymes and remained very high (ee >99%). Next, the concentration effect of the NAD⁺ on the yield of (*S*)-1-phenylethanol ((*S*)-1b) in the (1a) was studied and it was found that only a small amount is necessary to achieve the maximum yield of 1-phenylethanol (1b) (0.025 mol equivalence to the substrate) (Figure 2). The selectivity of the reduction also remained >99% ee even when only 0.0009 mole equivalence of NAD⁺ was employed in the reduction. The concentration effect of NADH, NADP⁺, NADPH were also studied and coenzyme concentration-yield profiles similar to that for NAD⁺ were obtained (data not shown).

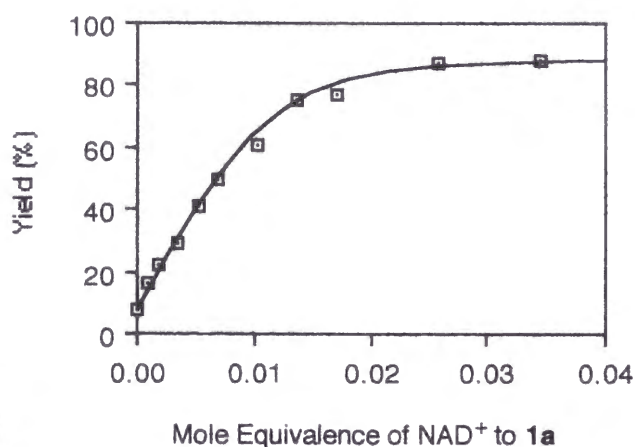


Figure 2. Effect of NAD⁺ on the reduction of 1a.

Reaction Conditions: 20 h at 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). 1a: 0.08 mmol, APG4: 10 mg, 2-propanol: 1.31 mmol. Yields and ee were determined by GC analyses, and ee was >99 %(*S*) for any amount of the coenzyme employed except when no coenzyme was employed.

Reducing Agents.

The reduction catalyzed by APG4 does not proceed without a reducing agent even in the presence of a coenzyme as shown in Table 1, which indicates that this system requires a reducing agent to recycle the oxidized coenzyme. The usage of glucose-6-phosphate,^{11a,20} glucose,^{11a,21} formate,²² ethanol,⁸ 2-propanol,^{9 - 12,15b,e} 2-butanol,¹³ *endo*-bicyclo[3.2.0]hept-2-en-6-ol,¹⁴ 2-hexanol,^{15e} and cyclopentanol^{15c,15d,15e} to regenerate the reduced form of coenzymes, and FMN,²³ and cyclohexanone²⁴ to regenerate the oxidized form of coenzymes have been reported. For the APG4 reduction system, the applicability of various alcohols as reducing agents in the (1a) was investigated, and the results are shown in Figure 3. Among the alcohols tested for their abilities as a reducing agent, 2-alkanols (2-propanol and racemic 2-pentanol) and cyclopentanol exhibited excellent

results for both yield and ee. Therefore, 2-alkanols and cyclopentanol were employed for further investigation to examine their concentration effect.

Differences in effectiveness between cyclopentanol and cyclohexanol can be explained on the basis of the higher strain energy of cyclohexanone than that of cyclopentanone. The easiness of the reduction of cyclohexanone compared with that of cyclopentanone was demonstrated in the relative rate (23 : 1) of the sodium borohydride reduction.²⁵ Cyclohexanone is easily reduced back to cyclohexanol competing with the reduction of the main substrate, but cyclopentanone is reduced much slower in the undesired direction. The difference in the effectiveness as a reducing agent between cyclopentanol and cyclohexanol stems from the difference in the relative reduction rate of their oxidized forms.

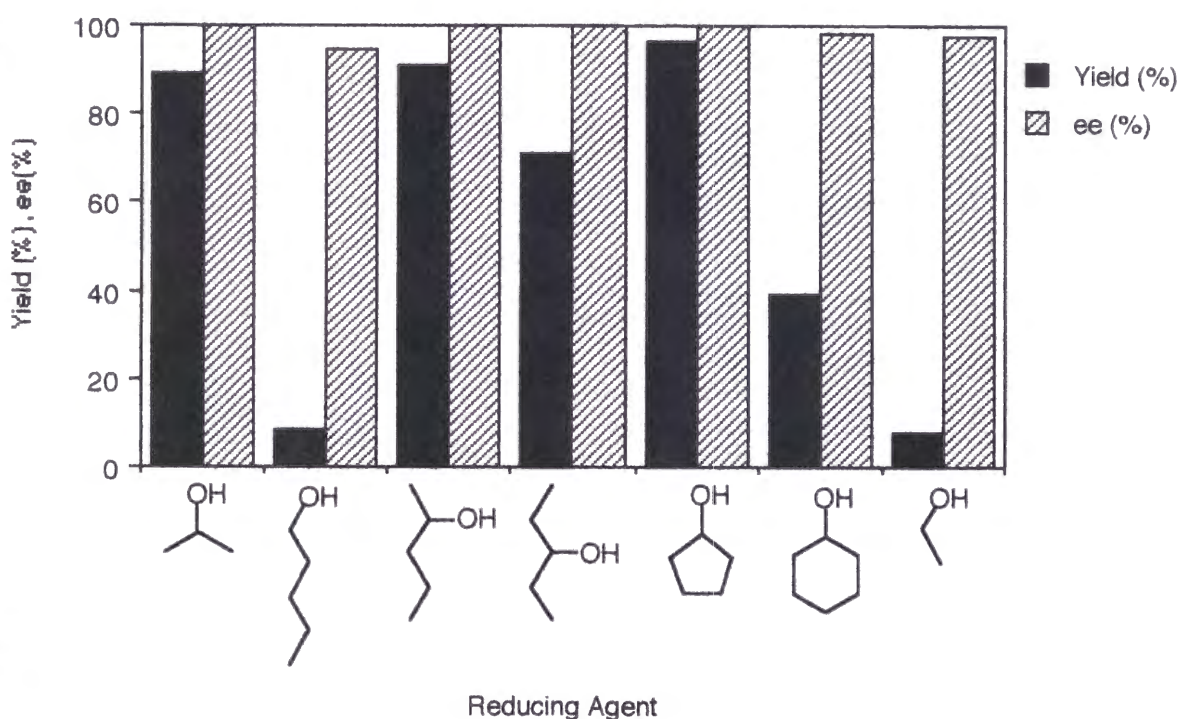


Figure 3. Effect of alcohols as reducing agents on the reduction of **1a**.

Reaction Conditions: 20 h at 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, reducing agent: 1.31 mmol. Yields and ee were determined by GC analyses.

Examination of the effect of the concentration of reducing agents clarified that a large excess of the reducing agents is necessary to maximize the yield, as shown in Figure 4. 2-Propanol and racemic 2-pentanol afford similar correlations in the yield - mole equivalence of the reducing agent relationship. All 2-alkanols from 2-propanol to 2-octanol were also as similarly effective as 2-

propanol or 2-pentanol (data not shown), whereas cyclopentanol worked a little better than 2-propanol and 2-pentanol. An excess amount of the reducing agent is necessary to obtain the product in high chemical yields since the product, (*S*)-1-phenylethanol (*(S)*-**1b**), is oxidized back to acetophenone (**1a**) by the same enzyme(s). (See Scheme 1) Even with the excess amount of a reducing agent, the yield of the reaction does not reach 100% since increases in the yield stopped when the equilibrium between the desired reaction (**1a**) and the undesired reverse reaction (oxidation of 1-phenylethanol (**1b**)) are reached. The undesired oxidation of 1-phenylethanol (**1b**) through a different route under aerobic conditions^{24,26} also prevents the yield from becoming quantitative.

Although 2-alkanols from 2-propanol to 2-octanol and cyclopentanol were effective for the reduction system, from the synthetic point of view, the usage of 2-propanol as a reducing agent is most advantageous due to its high volatility since a large amount of the remaining reducing agent has to be separated from the product during the work-up.

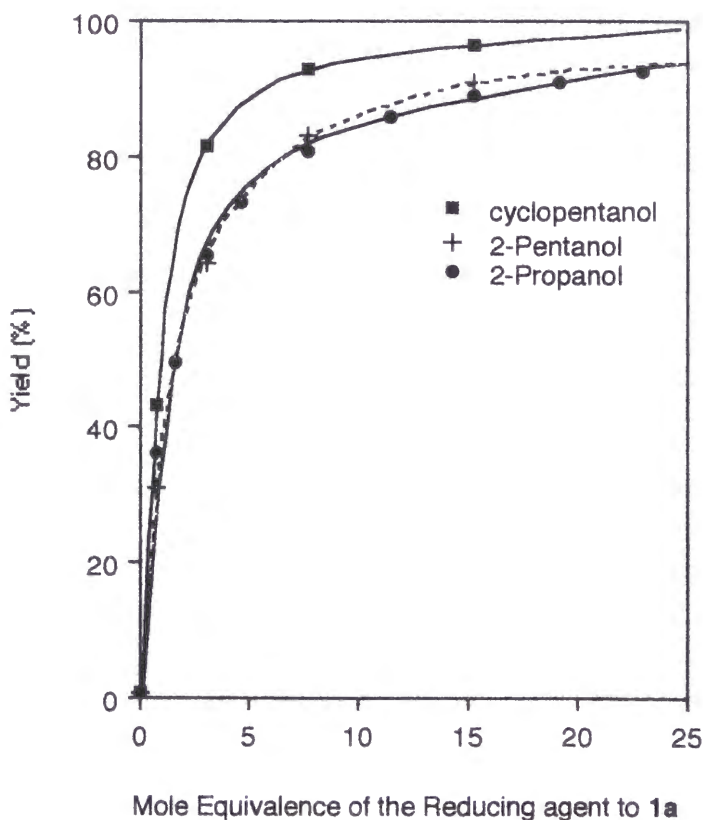


Figure 4. Effect of 2-alkanols and cyclopentanol as reducing agents on the reduction of **1a**.

Reaction Conditions: 20 h at 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol. Yields and ee were determined by GC analyses, and ee was >99 %(*S*) for any amount of reducing agent employed except when no reducing agent was employed.

Kinetic Parameters.

Using APG4 homogenized with MES buffer (pH 7.0, 0.1 M), the initial rates of the (**1a**) were measured as a function of the substrate concentration. The apparent kinetic parameters (K_m and V_{max}) were calculated *via* a Lineweaver-Burk plot to be 1.7 mM and 0.0085 mM•min⁻¹•mg⁻¹ of APG4 for K_m and V_{max} , respectively. K_m obtained herein for **1a** is comparable to the K_m (0.43 mM to a few hundred mM) of the dehydrogenase isolated from bakers' yeast for the α -keto ester reduction.^{16c}

Buffer.

The effect of the buffer used in the (**1a**) by APG4 on the reaction rate, yield and ee is shown in Table 2. It was found that various kinds of buffers at pH 7.0 can be used for this reaction. Although the reaction rate depends slightly on the kind of buffers used, little effect on the yield was observed. Importantly, the enantioselectivity of the reduction remained very high (>99% ee) regardless of the kind of buffers. However, the buffer actions must be present for the reaction to proceed because the reaction does not occur when ion exchanged water is used as a solvent.

Table 2. Effect of Kind of Buffers at pH 7.0 on the Catalytic Activity of APG4 in the Reduction of **1a**

Buffer	Rate ^a	Yield(%) ^b	ee(%) ^b
MES (2-(<i>N</i> -morpholino)ethanesulfonic acid - NaOH)	1.0	88	>99
KPB (Potassium Phosphate Buffer)	1.2	88	>99
HEPES (4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid - NaOH)	0.8	89	>99
MOPSO (β -hydroxy-4-morpholinepropanesulfonic acid - NaOH)	1.0	88	>99
TRIS (Tris(hydroxymethyl)aminomethane-HCl)	0.9	87	>99
Im (Imidazole-HCl)	0.4	63	>99
Ion exchanged water	0.0	0	-

Reaction Conditions: 30 °C at 130 rpm in a buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^a Relative initial rates of reduction were determined by GC analysis, where the reduction rate of **1a** in MES Buffer was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). ^b Determined by GC analyses at 20 h.

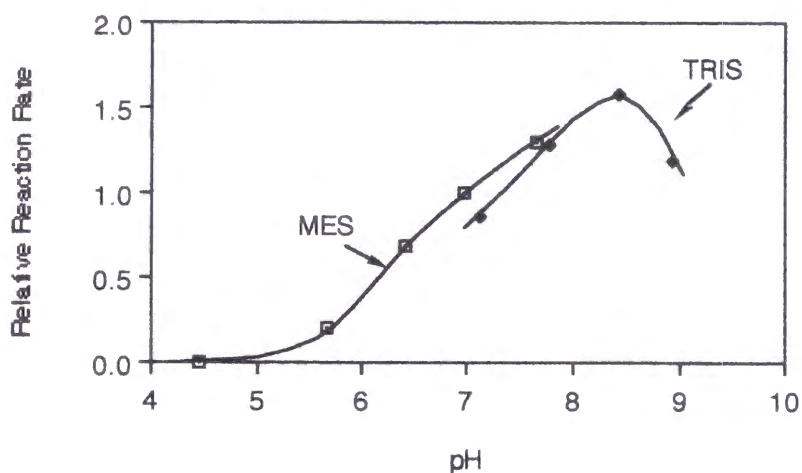
pH.

The effect of pH on the reaction rate, yield and ee was also examined. As shown in Figure 5, the optimum pH for the (**1a**) by APG4 is around 8.5, although it proceeds within a wide pH range from 5.7 to higher than 8.9. The rate limiting step of this reduction system is supposed to be the regeneration of the coenzyme from the oxidized form (NAD⁺) to the reduced form (NADH) rather

than the reduction of the acetophenone (**1a**) based on the optimum pH being 8.5 since the reduction of NAD⁺ is faster in basic conditions than in acidic conditions. Interestingly, the reaction rate depends on the pH value, but the effect of pH on the yield of 1-phenylethanol (**1b**) as shown in Table 3 is very small between a pH value of 6.4 and 8.9, because the yield is determined by the equilibrium between the oxidation and reduction as discussed above. The enantioselectivity of the reduction is not affected at all by the pH value between 5.7 and 8.9. The firmness of this excellent enantioselectivity which is not dependent on the pH value, kind of buffer, kind and amount of reducing agents and coenzymes, and amount of APG4 used, is a special feature of this reduction system.

Figure 5. Effect of pH Values on the Reaction Rate in the Reduction of **1a**.

Reaction Conditions: 30 °C at 130 rpm in a buffer (0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg,



NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. Relative reaction rates of reduction were determined by GC analysis, where the reduction rate of **1a** in MES Buffer at pH 7.0 was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min).

Reaction Temperature.

Figure 6 illustrates the time courses of the reaction at several reaction temperatures. The optimum reaction temperature was found to be 40 °C, whereas the reaction at 32 °C and 50 °C also proceeded smoothly, resulting in a high yield. However, at 60 °C, the reaction rate dropped to only one third of the rate at the optimum reaction temperature. Moreover, the reaction did not proceed any further after 4h.

The stereoselectivity of the reduction was not influenced by the reaction temperature (>99% ee was obtained for the all reaction temperatures studied). The excellent stereoselectivity, the peculiarity of this APG4 reduction system, was steady.

Table 3. Effect of pH on the Yield and Ee in the Reduction of **1a**

Buffer	pH	Yield(%) ^a	ee(%) ^a
MES	4.4	0	-
MES	5.7	41	>99
MES	6.4	86	>99
MES	7.0	88	>99
MES	7.7	88	>99
TRIS	7.1	85	>99
TRIS	7.8	87	>99
TRIS	8.4	87	>99
TRIS	8.9	86	>99

Reaction Conditions: 20 h at 30 °C at 130 rpm in MES or TRIS buffer (0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^aDetermined by GC analyses.

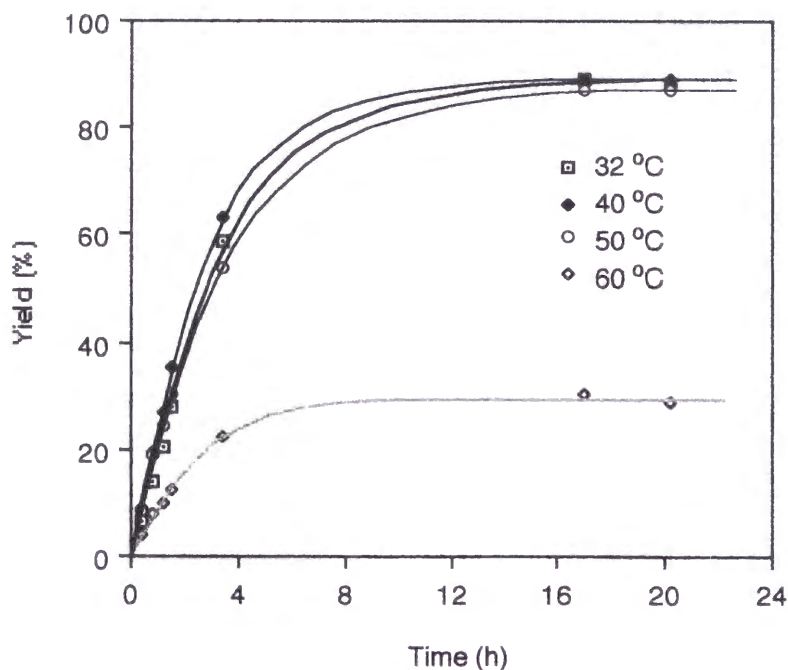


Figure 6. Effect of Reaction Temperature on Time Course of the APG4 Reduction of **1a**.

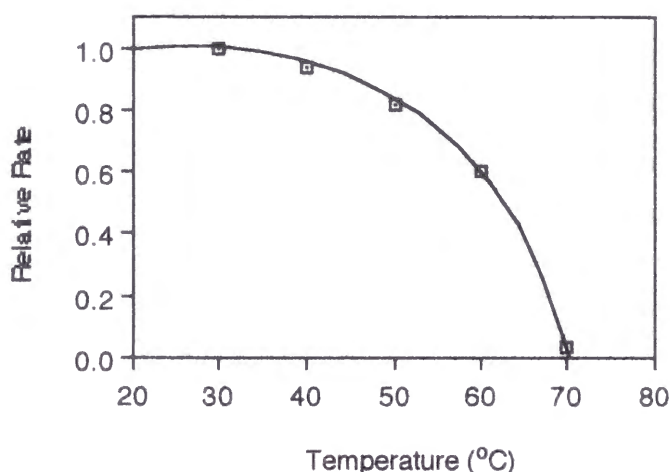
Reaction Conditions: at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. Yields and ee were determined by GC analyses. Ee was >99 %(*S*) at any temperature at 20 h.

Thermostability.

APG4 was incubated at various temperatures for 30 min at pH 7.0 and the residual activity was measured. As shown in Figure 7, APG4 was very stable at 30 °C in MES buffer and did not lose any activity. The activity of APG4 was retained at 94%, 82%, and 62% of the original after heating

at 40 °C, 50 °C, and 60 °C for 30 min, respectively. However, most of the activity was lost with heating at 70 °C. The selectivity of the reduction was not influenced by incubation below 60 °C prior to the reaction and was maintained >99% ee.

Figure 7. Thermostability of APG4.



APG4 was incubated in MES buffer (pH 7.0, 0.1 M, 3 mL) at various temperatures for 30 min, and the residual activity was measured. Reaction Conditions: 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. Relative reaction rates of reduction were determined by GC analysis, where the reduction rate of **1a** by APG4 without any incubation prior to the reaction to start was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). Ee was determined by GC analyses at 20 h to be 99% for all of incubation temperatures employed except the incubation temperature of 70 °C.

Preservability.

Preservability of biocatalysts is one of the most important factors in judging applicability of catalysts to organic synthesis. Accordingly, preservability of APG4 at 30 °C and -20 °C was investigated and the results are shown in Table 4. Most of the activity of APG4 was retained after 48 h at 30 °C, and 69% and 35% were retained after 10 days and 22 days at 30 °C, respectively. Amazingly, the enzyme activities were still preserved to a certain extent even after the storage of APG4 for more than 45 days at 30 °C. APG4 can also endure long term preservations in a refrigerator; 69% of the activity was preserved when the enzyme was stored at -20 °C for more than 2 years without special care. This proved that the observed stability and preservability of APG4 were remarkable.

The thermostability of APG4 during its preparation was also examined. APG4 was prepared using acetone at 25 °C instead of -20 °C, the usual temperature, and the activity was measured. APG4 prepared at 25 °C had 79% of the activity of usual APG4 in the (**1a**).

The enantioselectivity of the reduction remained very high (> 99%) even with the enzyme being

stored for 45 days at 30 °C, stored for 2 year at -20 °C or prepared at 25 °C.

Table 4. Preservability of APG4

Destabilization Conditions			
Temperature (°C)	Time	Residual Relative Rate ^a	ee(%) ^b
30	24 hours	0.99	>99
30	48 hours	0.92	>99
30	10 days	0.69	>99
30	22 days	0.35	>99
30	45 days	0.12	>99
-20	2 years	0.69	>99

APG4 was placed in air at 30 °C or -20 °C for the indicated time periods and their residual activities were measured. Reaction Conditions: 30 °C at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL). **1a**: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^a Relative initial rates of reduction were determined by GC analysis, where the reduction rate of **1a** by APG4 freshly prepared was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). ^b Determined by GC analyses at 20 h.

Residual Activity of APG4 after the (1a).

The residual activity of APG4 after the reaction of acetophenone (**1a**) was examined. After acetophenone (**1a**) was reduced by the APG4 system and when the yield of the reduction reached a maximum value at 14 h, additional acetophenone (**1a**) was employed in the reaction mixture and the time course of the reduction was taken to examine if the system still had some reducing power. Additional 2-propanol and/or NAD⁺ were also employed together with acetophenone (**1a**) to check the cases where APG4 is still active but the coenzyme, NAD⁺ has decomposed and/or an insufficient amount of a reducing agent (2-propanol) is left to reduce acetophenone (**1a**). As shown in Figure 8, when both of 2-propanol and NAD⁺ were added together with the additional acetophenone (**1a**), the initial rate of the reduction was 60% of that of the standard, but the yield of the reaction increased to the maximum value (88%). When only NAD⁺ was added together with acetophenone (**1a**), the rate was only 40% of that of the standard. The rate was only 25% and 20% of that of the standard when only 2-propanol was added together, and when none of the additional elements were added, respectively. From these results, it is concluded that the activity of APG4 is maintained at 60% even after the (**1a**), but NAD⁺ is decomposed after the reaction and an additional reducing agent, 2-propanol, is also necessary for the reduction of additional acetophenone (**1a**) to proceed smoothly.

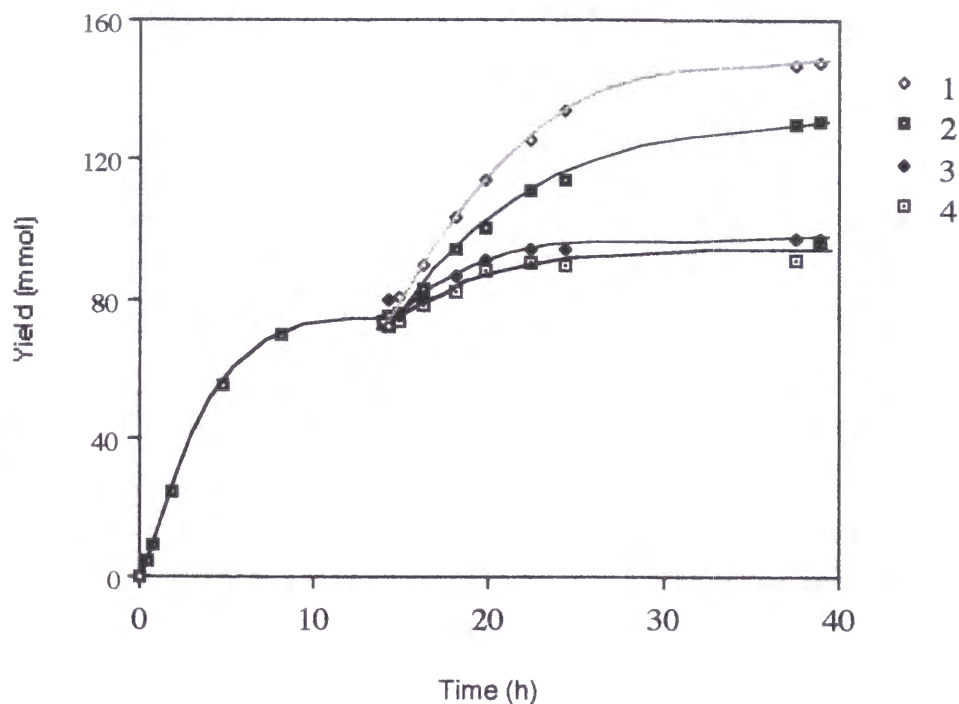


Figure 8. Residual Activity of APG4 after the reaction.

1a (0.08 mmol) was reduced under the reaction conditions: at 130 rpm in MES buffer (pH 7.0, 0.1 M, 3 mL), APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. At 14 h, additional **1a** (0.08 mmol) was added to the reaction mixture together with 1: 2-propanol (1.31 mmol) and NAD⁺ (0.007 mmol), 2: NAD⁺ (0.007 mmol), 3: 2-propanol (1.31 mmol), 4: none. Yields were determined by GC analyses. Ee was determined by GC analyses to be 99% for all the conditions at 39 h.

As a result of the thorough examination of the reaction conditions for APG4 reduction as well as the stability of APG4, it was found that the rate of APG4 reduction is dependent on the conditions of APG4 as well as the reaction conditions, the yield of the reaction changes only with a drastic change in the conditions, and the enantioselectivity remains very high (>99% ee) regardless of the reaction conditions. Similar experiments examining the reaction conditions for the APG4 reduction of β -keto esters were also conducted using ethyl 3-ketobutyrate or methyl 3-ketobutyrate as substrates. The yield was a little higher for the reduction of β -keto esters than that of acetophenone (**1a**), but analogous trends in the relationship between the yield and the conditions were observed. Enantioselectivity was also perfect for the reduction of β -keto esters regardless of the reduction conditions.

Mechanism

To elucidate the mechanism for stereoselective reduction by APG4, NAD(P)⁺ and cyclopentanol or 2-alkanols, enzymes were separated. As shown in the MonoQ-HR column chromatograph of the cell-free extract prepared from APG4 for the reduction of **1a** by NADPH (Figure 9), there were many enzymes present in APG4; one *R* selective enzyme was found, while the others showed *S* selectivity. The ee of 80% (*S*) in the reduction of **1a** with the cell-free extract and NADPH (Table 5, Entry 1) also showed that many enzymes in APG4 with different selectivities participated in the reduction.

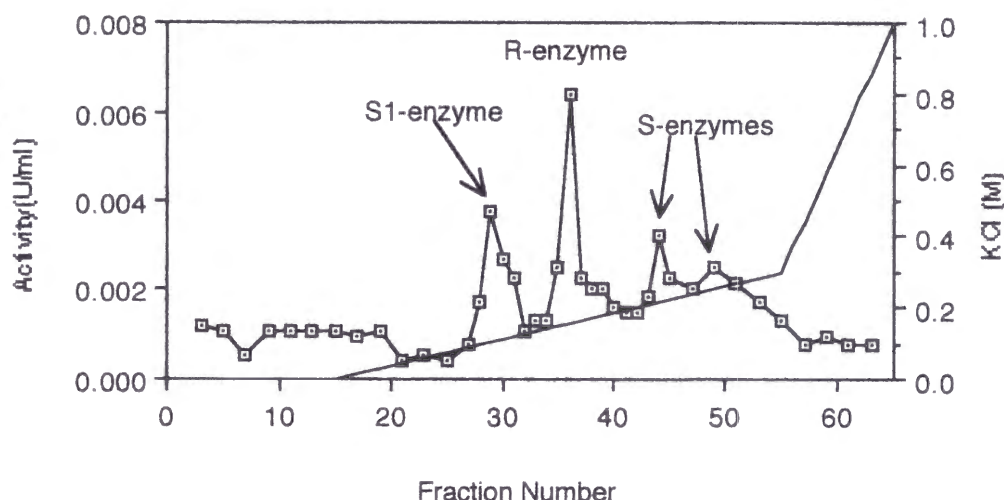


Figure 9 MonoQ-HR column chromatograph of the cell-free extract.

Activity for the reduction of **1a** by NADPH is shown. Cell-free extract was obtained by centrifugation of sonic-disintegrated APG4 in HEPES buffer.

Table 5 Enzymatic reduction of **1a**.

Entry	Enzyme	Coenzyme	Cyclopentanol	Yield(%)	Ee(%)	Config.
1	Cell-free extract	NADPH	-	59	80	<i>S</i>
2	Cell-free extract	NADP ⁺	+	77	>99	<i>S</i>
3	S1-enzyme	NADPH	-	5	>99	<i>S</i>
4	S1-enzyme	NADP ⁺	+	60	>99	<i>S</i>
5	R-enzyme	NADPH	-	69	92	<i>R</i>
6	R-enzyme	NADP ⁺	+	12	92	<i>S</i>
7	S1-enzyme + R-enzyme	NADPH	-	64	22	<i>R</i>
8	S1-enzyme + R-enzyme	NADP ⁺	+	59	>99	<i>S</i>

Enzymes isolated and rechromatographed by FPLC (MonoQ-HR) were used for the reactions. Yield, ee and an absolute configuration of the reduction of **1a** (0.0025 mmol) by each fraction with NADPH or NADP⁺ and cyclopentanol were determined using a chiral GC-column (Chirasil-DEX CB; 25 m; He 2 mL/min).

When the S1-enzyme and the R-enzyme were used separately for the reduction of **1a** in the presence of NADPH, (*S*)-**1b** and (*R*)-**1b** with high ee were obtained, respectively (Table 5, Entry 3, 5). (*S*)-**1b** was also obtained by the catalysis of the S1-enzyme in the presence of NADP⁺ and cyclopentanol (Table 5, Entry 4), which indicates that the S1-enzyme can recycle NADP⁺ using cyclopentanol. (The formation of cyclopentanone was also confirmed by GC analysis.) A similar result was also obtained using isopropanol instead of cyclopentanol (data not shown). However, when R-enzyme was used for the reduction of **1a** in the presence of NADP⁺ and cyclopentanol, the yield of the reduction was low (Table 5, Entry 6) which means that the R-enzyme can not use cyclopentanol to produce NADPH. ((*S*)-**1b** was produced with 12 % yield due to the contamination of the S1-enzyme in the R-enzyme fraction.)

When both of the S1-enzyme (0.0011 unit) and the R-enzyme (0.0059 unit) were added to the reaction mixture for the reduction of **1a**, an almost racemic product (*R/S*)-**1b** was obtained in the presence of NADPH (Table 5, Entry 7), while enantiomerically pure (*S*)-**1b** was obtained in the presence of NADP⁺ and cyclopentanol (Table 5, Entry 8). The dramatic difference in the selectivities can be explained as follows; when NADPH is added to the reaction mixture, both enzymes can use NADPH to reduce **1a**, affording a mixture of both enantiomers (Figure 10(a)). However, when NADP⁺ and cyclopentanol instead of NADPH are used for the reduction, the catalytic cycle of the S1-enzyme turns but that of the R-enzyme does not; the S1-enzyme can obtain NADPH by reducing NADP⁺ with coupled oxidation of cyclopentanol, but the R-enzyme can not obtain the reduced form of the coenzyme, NADPH (Figure 10(b)). Moreover, the catalytic cycle to produce (*S*)-**1b** is much more efficient than the theoretical cycle involving both the S1-enzyme and the R-enzyme producing (*R*)-**1b** shown in Figure 10 (c). In fact, ee of the reduction of **1a** in the presence of the S1-enzyme, R-enzyme, NADP⁺ and cyclopentanol is >99% (*S*) (Table 5, Entry 8). The reduction of **1a** by the cell-free extract with NADP⁺ and cyclopentanol also affords optically pure (*S*)-**1b** (Table 5, Entry 2). Based on these facts, it is supposed that the S1-enzyme oxidizes cyclopentanol to produce NADPH and uses it for the reduction of **1a** before transferring it to the R-enzyme. Therefore, the addition of cyclopentanol to the reduction system of APG4 and NADP⁺ increases the activity of only the S1-enzyme but not the R-enzyme, resulting in stereoselective reduction.

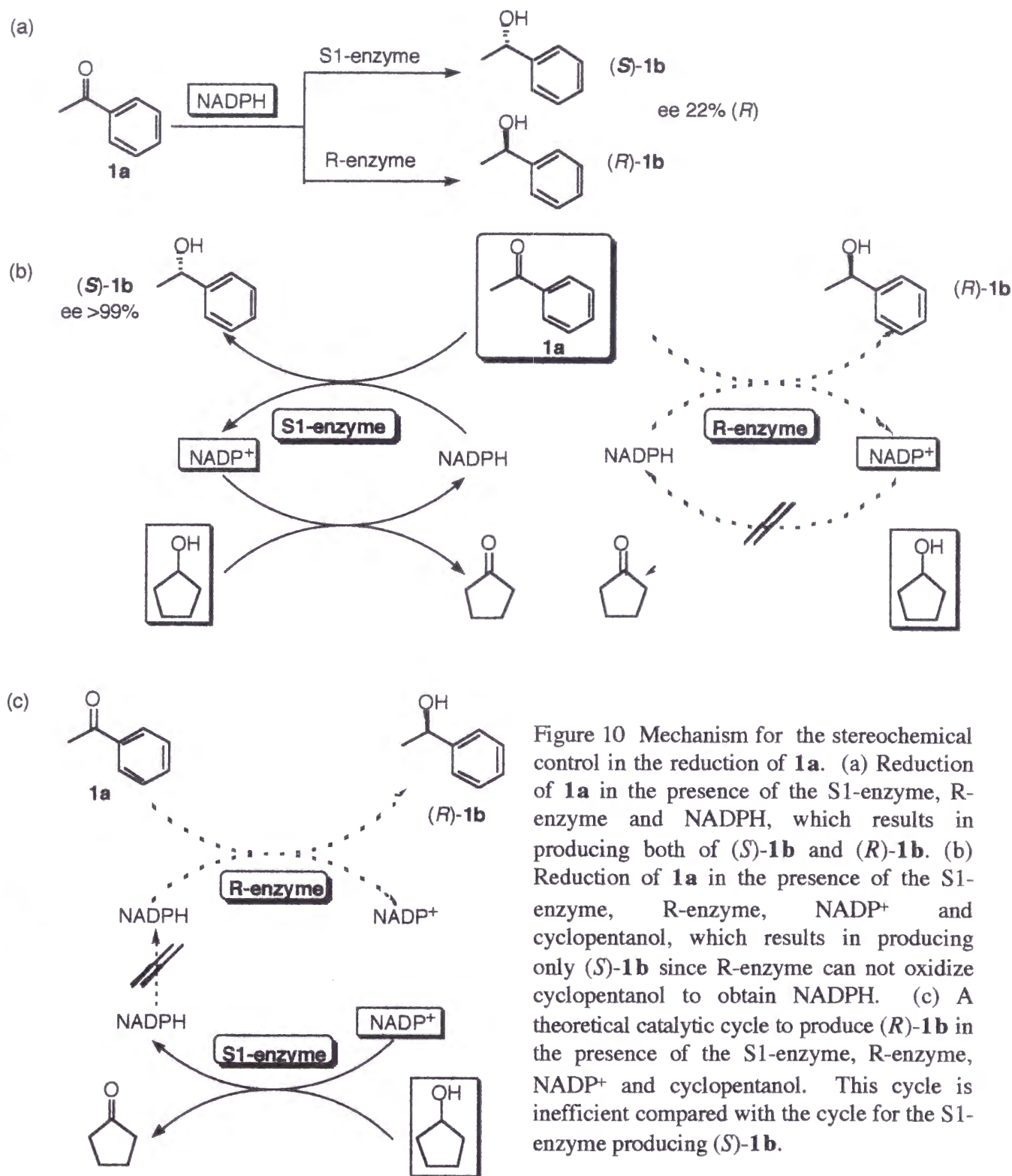


Figure 10 Mechanism for the stereochemical control in the reduction of **1a**. (a) Reduction of **1a** in the presence of the S1-enzyme, R-enzyme and NADPH, which results in producing both of (S)-**1b** and (R)-**1b**. (b) Reduction of **1a** in the presence of the S1-enzyme, R-enzyme, NADP⁺ and cyclopentanol, which results in producing only (S)-**1b** since R-enzyme can not oxidize cyclopentanol to obtain NADPH. (c) A theoretical catalytic cycle to produce (R)-**1b** in the presence of the S1-enzyme, R-enzyme, NADP⁺ and cyclopentanol. This cycle is inefficient compared with the cycle for the S1-enzyme producing (S)-**1b**.

III. Reduction of Unfluorinated Ketones

Aromatic ketones, β -keto esters, and simple aliphatic ketones were reduced with excellent selectivity to the corresponding (*S*)-alcohols by using the acetone powder of *Geotrichum candidum*. This method is very convenient for the synthesis of optically pure alcohols in the gram scale.

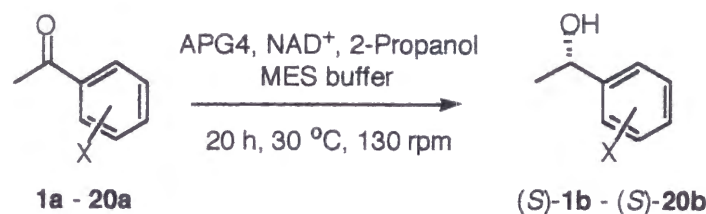
The present reduction system has a great synthetic value due to the extremely wide substrate specificity; aromatic ketones, β -keto esters, and simple aliphatic ketones are reduced with excellent selectivity to the corresponding (*S*)-alcohols.

The incredibly high enantioselectivity of the APG4 reduction system is proven by the derivatives with substituent(s) at the aromatic ring. Ortho, meta, or para substituted fluoro, chloro, bromo, methyl, methoxy, and trifluoromethyl acetophenone (**2a** - **19a**) and pentafluoroacetophenone (**20a**) were reduced by the APG4 reduction system and the results are listed in Table 6. The excellent enantioselectivity was hardly affected at all by the type or position of the substituent(s). An enantioselectivity of more than 99% ee was obtained for the reduction of all the acetophenone derivative tested except that for *o*-trifluoromethylacetophenone (**17a**) being 97% ee. Meanwhile, the yield of the reduction depends on the position of the substituent. Generally, the resulting alcohols were obtained in quantitative yields when there was a substituent at the ortho position. The yield of the meta substituted acetophenone was slightly lower than that of ortho substituted acetophenone, and a para substituent decreased the yield. This trend is not affected by the inductive effect of the substituent(s) since the fluoro or chloro substituents have the same effect on the yield as the methyl group. The plausible reason for the effect of the position of the substituent on the yield is that the yield is determined by the equilibrium between the reduction and undesired oxidation as described in the reducing agent section. It is known that this microbe oxidizes para substituted (*S*)-phenylethanol smoothly, but ortho substituted phenylethanol are not oxidized at all²⁷ meaning the yield of the reduction increases in the order of para, meta, and ortho substituted acetophenone.

The extremely high optical purities (ee >99%) of the resulting alcohols together with the high chemical yields in the reduction of most of the acetophenone derivatives tested make this method attractive for preparative purpose. In fact, the reduction in a gram scale proceeds smoothly without any loss in ee and the isolation of the product alcohol with high yields was possible as illustrated in the reduction of *o*-bromoacetophenone (**8a**, 2.6 g) to (*S*)-1-(*o*-bromophenyl)ethanol ((*S*)-**8b**) giving an 89% yield (2.3 g, >99% ee). Importantly, any organic chemist will be able to reproduce this result because the high selectivity of this reduction system is very steady and independent of small changes

in the experimental conditions such as scale of the reaction, pH, reaction temperature, *etc.*.

Table 6. Reduction of Aryl Methyl Ketones by APG4

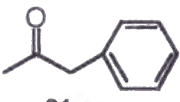
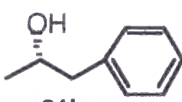
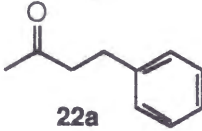
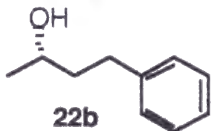
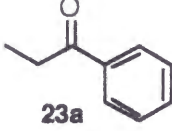
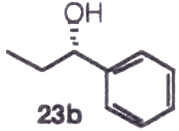
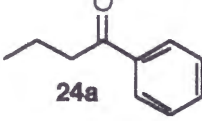
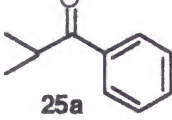
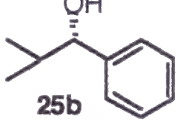
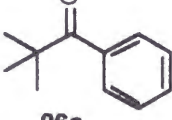
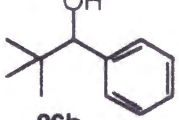
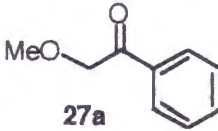
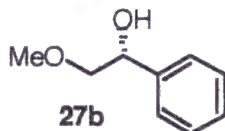
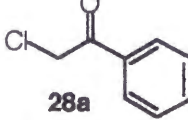
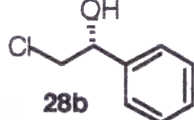


X	Yield(%) ^{a, b}	ee(%) ^a	Config. ^c
H (1)	89(74)	>99	S
<i>o</i> -F (2)	>99(94)	>99	S
<i>m</i> -F (3)	95(90)	>99	S
<i>p</i> -F (4)	74(60)	>99	S
<i>o</i> -Cl (5)	>99(94)	>99	S
<i>m</i> -Cl (6)	95(91)	99	S
<i>p</i> -Cl (7)	62(80) ^d	>99	S
<i>o</i> -Br (8)	97(89)	>99	S
<i>m</i> -Br (9)	92(85)	>99	S
<i>p</i> -Br (10)	95(66)	>99	S
<i>o</i> -Me (11)	96(73)	>99	S
<i>m</i> -Me (12)	86(83)	>99	S
<i>p</i> -Me (13)	78(70)	>99	S
<i>o</i> -MeO (14)	84(85) ^d	>99	S
<i>m</i> -MeO (15)	90(83)	>99	S
<i>p</i> -MeO (16)	29(21)	>99	S
<i>o</i> -CF ₃ (17)	6(13) ^d	97	S
<i>m</i> -CF ₃ (18)	96(83)	>99	S
<i>p</i> -CF ₃ (19)	73(85)	>99	S
1',2',3',4',5'-F ₅ (20)	62(80)	>99	S

^a Determined by GC analyses. Reaction Conditions: MES buffer (pH 7.0, 0.1 M): 3 mL, substrate: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^b Isolated yield in parenthesis. See experimental section for the reaction conditions. ^c Determined as described in the experimental sections. ^d Higher isolated yield was obtained than GC yield with more enzyme or coenzyme, longer reaction time, or the reaction under argon atmosphere.

Reduction by APG4 of several aromatic ketones having different length alkyl chains demonstrates the scope and limitations of the substrate specificity (Table 7). Phenyl moiety of

Table 7. Reduction of Aromatic Ketones by APG4

Substrate	Product	Yield(%) ^{a, b}	ee(%) ^a	Config. ^c
 21a	 21b	96(78)	>99	<i>S</i>
 22a	 22b	93(95) ^d	>99	<i>S</i>
 23a	 23b	41(25)	>99	<i>S</i>
 24a	no reaction	0	-	-
 25a	 25b	12(11)	99	<i>S</i>
 26a	 26b	1	-	-
 27a	 27b	8(5)	>99	<i>R</i>
 28a	 28b	80(49)	98	<i>R</i>

^a Determined by GC, HPLC or NMR analyses. Reaction Conditions: MES buffer (pH 7.0, 0.1 M): 3 mL, substrate: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol.

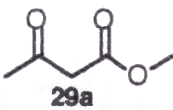
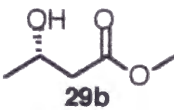
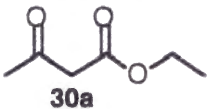
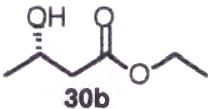
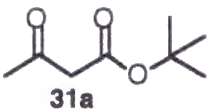
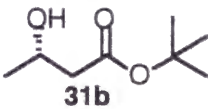
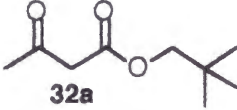
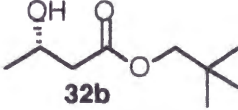
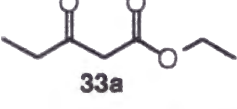
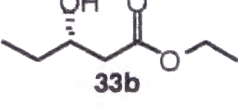
^b Isolated yield in parenthesis. See experimental section for the reaction conditions. ^c Determined as described in the experimental sections. ^d Higher isolated yield was obtained than GC yield with more enzyme or coenzyme, longer reaction time, or the reaction under argon atmosphere.

acetophenone can be replaced by a benzyl (**21a**) or even by a 2-phenylethyl (**22a**) group with slightly better results of chemical yield than the (**1a**) without a decrease in enantioselectivity.

However, when the methyl moiety of acetophenone is replaced by an ethyl (**23a**), isopropyl (**25a**) or methoxymethyl (**27a**) group, the yield dramatically decreases, although the enantioselectivity remained high (>99% ee), and when the alkyl chain is elongated to a propyl (**24a**) or enlarged to a *t*-butyl (**26a**) group, the reaction scarcely proceeds. α -Chloroacetophenone (**28a**) was also reduced by the APG4 system, and (*R*)- α -chloro-1-phenylethanol (**28b**) was obtained with 80% yield and 98% ee. This chiral alcohol can be converted to various compounds via epoxide.²⁸

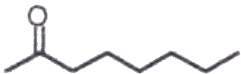
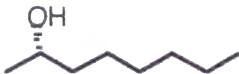





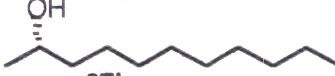
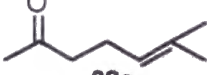

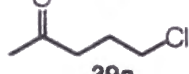
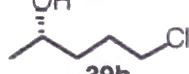
The versatility of the APG4 reduction system is further exemplified by the β -keto esters as substrates. The results of the reduction of 3-ketobutyrate (**29a** - **32a**) and a 3-ketopentanoate (**33a**) are shown in Table 8. 3-Ketobutyrate with ester moiety of any of methyl (**29a**), ethyl (**30a**), *t*-butyl (**31a**), or neopentyl (**32a**) are reduced to (*S*)-hydroxyl esters (**29b** - **32b**) with >99% ee quantitatively. The yield and ee remained >99% regardless of the ester moiety. Ethyl 3-ketopentanoate (**33a**) can also be reduced to *S*-hydroxyl ester (**33b**) with >99% ee in 72% yield. The present reduction system is certainly suitable for the reduction of 3-ketobutyrate and 3-ketopentanoates.

Table 8. Reduction of β -Keto esters

Substrate	Product	Yield (%) ^{a, b}	ee(%) ^a	Config. ^c
		>99	>99	<i>S</i>
		>99(59)	>99	<i>S</i>
		>99	>99	<i>S</i>
		>99(74)	>99	<i>S</i>
		72(52)	>99	<i>S</i>

^a Determined by GC analyses. Reaction Conditions: MES buffer (pH 7.0, 0.1 M): 3 mL, substrate: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^b Isolated yield in parenthesis. See experimental section for the reaction conditions. ^c Determined as described in the experimental sections.

Table 9. Reduction of Aliphatic Ketones by APG4

Substrate	Product	Yield(%) ^{a, b}	ee(%) ^c	Config. ^d
 34a	 34b	87(62)	>99	<i>S</i>
 35a	 35b	87(77)	>99	<i>S</i>
 36a	 36b	85(49)	>99	<i>S</i>
 37a	 37b	60(61) ^e	>99	<i>S</i>
 38a	 38b	90(76)	99	<i>S</i>
 39a	 39b	92(40)	99	<i>S</i>

^a Determined by GC analyses. Reaction Conditions: MES buffer (pH 7.0, 0.1 M): 3 mL, substrate: 0.08 mmol, APG4: 10 mg, NAD⁺: 0.007 mmol, 2-propanol: 1.31 mmol. ^b Isolated yield in parenthesis. See experimental section for the reaction conditions. ^c Determined by GC analyses after acetylation of the product except for **38b**. ^d Determined as described in the experimental sections. ^e Higher isolated yield was obtained than GC yield with more enzyme or coenzyme, longer reaction time, or the reaction under argon atmosphere.

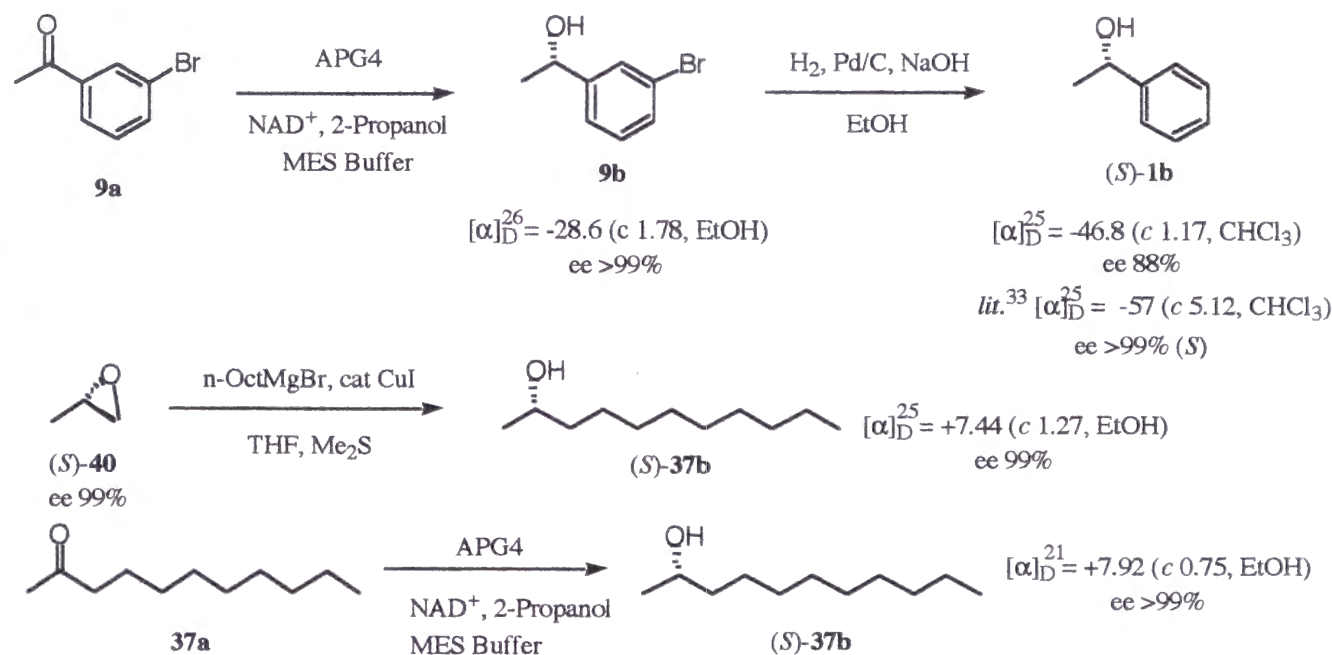
In the recent development of the catalyst for the asymmetric synthesis, efficient catalysts for the enantioselective reduction of functionalized ketones have been reported²⁹ but the asymmetric reduction of small aliphatic ketones remains a major challenge in organic chemistry.^{9a} It has been confirmed that the functional groups such as aromatic ring or ester moiety are not necessary to accomplish reduction by the APG4 system with high selectivity or reactivity. Simple aliphatic ketones from 2-octanone to 2-undecanone (**34a** - **37a**), 6-methyl-5-heptene-2-one (**38a**), and 5-chloro-2-pentanone (**39a**) were reduced by the APG4 system to the corresponding (*S*)-2-alkanols (**34b** - **39b**) giving high yields with 99% ee. The present reduction system is beneficial for the reduction of aliphatic ketones over a nonenzymatic system by which, for example, in the reduction of 2-octanone (**34a**), 82% ee is the highest to the best of our knowledge.³⁰ Moreover, the reduction of

2-octanone (**34a**) gives only 24% ee for the product alcohol with BINAL-H, the well known "super" catalyst for hydrogenolysis.^{29b} In contrast to these low selectivities, the reduction of 2-octanone (**34a**) by this present APG4 system affords (*S*)-alcohol (**34b**) with >99% ee.

This system can also reduce aliphatic ketones with functional groups such as olefin or chloride giving a high yield with 99% ee. For example, the reduction of 6-methyl-5-heptene-2-one (**38a**) by the APG4 system proceeded smoothly and afforded (*S*)-(+)-sulcatol (**39b**), the aggregation pheromone produced by males of *Gnathotrichus sulcatus*,³¹ in one step. The reduction of 5-chloro-2-pentanone (**39a**) was also successful and afforded (*S*)-5-chloro-2-pentanol (**39b**) giving high yields with 99% ee. This chiral alcohol is an excellent bifunctional building block that may be conveniently employed for synthesis of natural products containing chiral carbinol centers.^{9b} For example, (*S*)-**39b** is used as a chiral building block for the total synthesis of (+)-(*S,S*)-(cis-6-methyltetrahydropyran-2-yl)acetic acid,^{9b} a naturally occurring heterocycle that has been isolated from the perfume material civet, a glandular secretion of the civet cat (*Viverra civetta*).³²

Determination of Absolute Configurations.

Scheme 2



Absolute configuration of (-)-1-(*m*-bromophenyl)ethanol (**9b**) was determined by converting optically pure **9b** obtained from the APG4 reduction of *m*-bromoacetophenone (**9a**) to optically active 1-phenylethanol (**1b**) by Pd/H₂ to compare the sign of optical rotation with that of the literature

value.³³ Debromination of chiral **9b** proceeded giving moderate yields accompanied with a little racemization as shown in the Scheme 2. As a result, chiral **9b** obtained by APG4 reduction was determined to have an *S* configuration.

Absolute configuration of 2-undecanol (**37b**) was determined by synthesizing the authentic (*S*)-**37b** of which the sign of optical rotation was compared with that of the APG4 reduction product. Authentic (*S*)-**37b** was readily synthesized from (*S*)-propylene oxide (**40**) through the Grignard reaction with *n*-octylmagnesium bromide in the presence of copper iodide and dimethyl sulfide as shown in the Scheme 2. As a result, chiral **37b** obtained by APG4 reduction was determined to have an *S* configuration.

IV. Reduction of Fluorinated Ketones

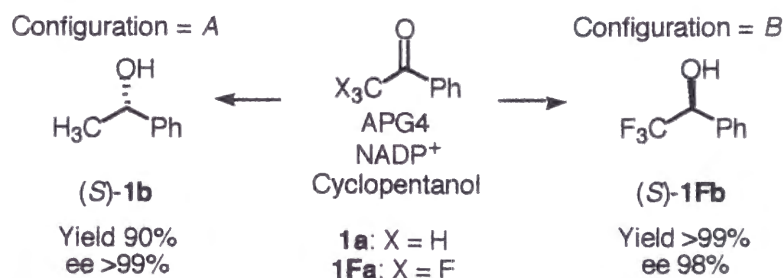
Reduction of methyl ketones by the acetone powder of *Geotrichum candidum* (APG4) afforded (*S*)-alcohols in excellent ee, whereas the reduction of trifluoromethyl ketones gave the corresponding alcohols of the opposite configuration also in excellent ee. The replacement of the methyl moiety with a trifluoromethyl group alters both the bulkiness and electronic properties, whose effect on the stereoselectivity was examined. No inversion in stereochemistry was observed in the reduction of hindered ketones such as isopropyl ketone, while the stereoselectivity was inverted in the reduction of ketones with electron-withdrawing atoms such as chlorine.

The mechanism for the inversion in stereochemistry was investigated by enzymatic studies. Several enzymes with different stereoselectivities were isolated; one of them catalyzed the reduction of methyl ketones, and another with the opposite stereoselectivity catalyzed the reduction of trifluoromethyl ketones.

Furthermore, APG4 as well as the isolated enzyme were applied to the reduction of fluorinated ketones on a preparative scale which resulted in the synthesis of chiral fluorinated alcohols with excellent ee.

Research on optically active fluorinated compounds for ferroelectric liquid crystals or bioactive fluorinated compounds has received much attention in the last few years because of the unique physical and biological features induced by fluorine atoms.¹⁸ As the demand for optically active fluorinated compounds increases, the importance of the development of asymmetric synthetic methods for fluorinated building blocks grows. Therefore, the substrate specificity of the APG4 system has been expanded to include fluorinated ketones. Surprisingly, different configurational alcohols were obtained by subjecting the trifluorinated ketone (trifluoroacetophenone, **1Fa**) and its unfluorinated analogue (acetophenone, **1a**) to the same reduction system as shown in Scheme 3. The absolute configuration of (*S*)-**1b** and (*S*)-**1Fb** are opposite by definition, so for convenience, the notation "A" and "B" is used to describe the absolute configuration of (*S*)-**1b** and (*S*)-**1Fb**, respectively, as shown in Scheme 3.

Scheme 3



A search of the literature uncovered several reports comparing the methyl and trifluoromethyl group for the ability to direct enantioselection in biocatalytic³⁴ and organometallic³⁵ reactions. Kitazume *et al* investigated the effect of fluorination on the stereoselectivity of bakers' yeast reduction^{34a-b} and Lipase MY-catalyzed hydrolysis.^{34a,c-e} In some cases when biocatalysts are used, the stereoselectivities are affected by the fluorination, but a completely different stereoselectivity between methyl and trifluoromethyl substrates as in our case (>99% ee for **1a**, 98% ee for **1Fa**) is not found. The inversion in stereochemistry in organometallic reactions is also observed for the reduction of **1a** and **1Fa** by (-)-DIP-Chloride and CBS/catecholborane but not by R-Binal-H, LAH-sulfamide, NB-Enantride and K-Glucoride. The mechanism of the inversion for the biocatalytic reactions has not been thoroughly investigated whereas that for the organometallic reaction has been explained by structural and mechanistic analyses.^{35a-f}

In this section, the factor which causes the inversion in biocatalytic reactions is investigated by changing the substituents at the α -position of acetophenone systematically as well as by enzymatic studies. Moreover, besides having an interesting stereochemistry, this system is valuable for synthetic purposes. Various chiral fluorinated alcohols can be synthesized using the APG4 system as well as by the separated enzyme. Monofluoromethyl and difluoromethyl ketones are reduced to both enantiomers by modifying the experimental conditions and choosing either the isolated enzyme or APG4.

Stereochemistry of the APG4 Reduction System

When 2,2,2-trifluoroacetophenone (**1Fa**) was subjected to reduction by the APG4 system, the corresponding alcohol, (*S*)-**1Fb**, was obtained in 98% ee, whereas the (**1a**), an unfluorinated analogue, afforded (*S*)-**1b** in excellent ee as shown in Scheme 3. Surprisingly, different configurational alcohols were obtained by subjecting the trifluorinated ketone and its unfluorinated analogue to the same reduction system, and the stereoselectivities were excellent for both substrates. The experimental conditions were fixed throughout the study to investigate the stereochemistry of the APG4 system as described in the experimental section.

The inversion in stereochemistry between the reduction of methyl and trifluoromethyl ketones was also observed in various substrates as shown in Table 10. When the phenyl group of acetophenone was replaced with a *p*-substituted phenyl, benzyl, thienyl, or even alkyl group, the inversion was observed. Therefore, the stereochemistry of the APG4 reduction system is determined from the fluorination pattern of the α -position; the configuration of the product is "A" for the reduction of methyl ketones and "B" for trifluoromethyl ketones.

Table 10. Reduction of Methyl and Trifluoromethyl Ketones

$ \begin{array}{ccccc} \text{H}_3\text{C}-\text{CH}(\text{OH})-\text{R} & \xleftarrow{\quad} & \text{X}_3\text{C}-\text{C}(=\text{O})-\text{R} & \xrightarrow{\quad} & \text{F}_3\text{C}-\text{CH}(\text{OH})-\text{R} \\ \text{Configuration} = A & & \text{APG4} & & \text{Configuration} = B \\ & & \text{NADP}^+ & & \\ & & \text{Cyclopentanol} & & \end{array} $						
X = H				X = F		
R	Substrate	Yield ^a (%)	ee ^{a,b} (%)	Substrate	Yield ^a (%)	ee ^a (%)
Ph	1a	90	>99 (<i>S</i>)	1Fa	>99	98 (<i>S</i>)
<i>p</i> -Chlorophenyl	7a	83	95 (<i>S</i>)	7Fa	91	>99 (<i>S</i>)
<i>p</i> -Bromophenyl	10a	94	91 (<i>S</i>)	10Fa	82	>99 (<i>S</i>)
Benzyl	21a	95	>99 (<i>S</i>)	21Fa	55	97 (<i>S</i>)
2-Thienyl	41a	45	>99 (<i>S</i>)	41Fa	>99	>99 (<i>R</i>)
3-Thienyl	42a	69	>99 ^c	42Fa	>99	>99 ^c
Hexyl	34a	97	98 (<i>S</i>)	34Fa	>99	96 (<i>S</i>)
Heptyl	35a	88	>99 (<i>S</i>)	35Fa	>99	96 (<i>S</i>)
Octyl	36a	87	>99 (<i>S</i>)	36Fa	>99	>99 (<i>S</i>)
Nonyl	37a	84	>99 (<i>S</i>)	37Fa	>99	98 (<i>S</i>)

The reaction conditions are described in the experimental section. ^a Determined by GC analysis. ^b Higher ee can be obtained when NAD⁺ and 2-propanol are used as a coenzyme and a reducing agent as described above. ^c The absolute configuration is not determined.

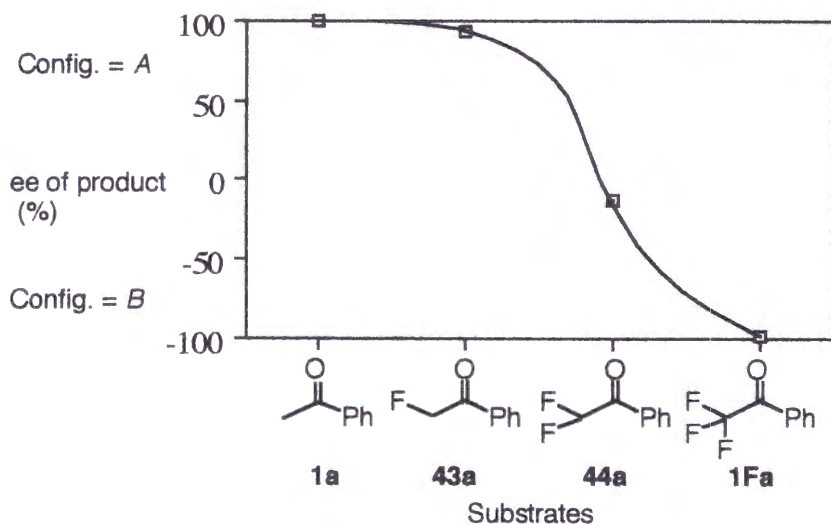


Figure 11. Effect of fluorination at the α -position of acetophenone on the stereoselectivity in the reduction by the APG4 system.

Next, monofluoroacetophenone (**43a**) and difluoroacetophenone (**44a**) were reduced by the APG4 system. The reduction proceeded quantitatively for both substrates. As expected, the stereoselectivity shifted from "A" to "B" according to the number of fluorine substituents at the α -

position as shown in Figure 11. The inversion is certainly caused by the fluorine substitution.

Since the increase in the number of fluorines at the α -position alters the bulkiness and electronic property, both effects on the stereoselectivity were studied to investigate the reaction mechanism. At first, the effect of bulkiness of the α -position was examined since in effective radius,^{18a,36} a trifluoromethyl moiety (2.2 Å) is larger than a phenyl moiety (1.62 Å) and similar to an isopropyl moiety (2.2 Å) as derived from rotational barriers in biphenyls. In the case of (-)-DIP-Chloride reported by Ramachandran *et al*, the stereochemical inversion caused by the substitution of methyl with trifluoromethyl is also observed on the substitution with *tert*-butyl.^{35a,b} Moreover, Corey's report of X-ray crystallographic studies of a set of trihalomethyl ketones indicates that the carbonyl oxygen is displaced significantly toward the trihalomethyl group, which acts more effectively as a larger group on the carbonyl carbon than *tert*-butyl or adamantyl.^{35c,d} Therefore, the stereoselectivity of the APG4 reduction of an ethyl ketone (**23a**), isopropyl ketone (**25a**), and *tert*-butyl ketone (**26a**) was examined. The results are shown in Table 11. No inversion in stereochemistry was observed in the reduction of hindered ketones by the APG4 system, although the yield of the reduction decreased according to the bulkiness. Therefore, the inversion is not merely caused by the bulkiness of the trifluoromethyl group.

Fluorine is the most electronegative atom and the electronic effect of trifluoromethyl group is magnificent. Accordingly, the electronic effect was examined by substituting an electronegative group at the α -position; the stereoselectivities of the reduction of mono (**28a**), di (**45a**), and trichloroacetophenone (**46a**), are shown in Figure 12. A similar trend in the shift of stereoselectivity from "A" to "B" was observed when two chlorine atoms were incorporated. However, the yield of the reduction of chloroacetophenones was much lower than that of corresponding fluoroacetophenones, and trichloroacetophenone (**46a**) was not reduced at all. The reduction of chlorodifluoroacetophenone (**47a**) was also "B" selective, with a quantitative yield. Methyl pentafluorophenyl ketone (**20a**) was reduced under the same conditions (NADP⁺ and cyclopentanol) with a slight loss of stereoselectivity (97% ee "A") (>99% ee "A" when NAD⁺ and 2-propanol are used); the shift by the electronegative substituent was observed even when it was on the other side of the carbonyl moiety.

Table 11. Effect of Bulkiness of the Group Adjacent to the Carbonyl Moiety on Stereoselectivity in the Reduction by the APG4 System

$$\begin{array}{ccc}
 \text{R}_\alpha\text{-C(=O)-Ph} & \xrightarrow[\text{Cyclopentanol}]{\text{APG4, NADP}^+} & \text{R}_\alpha\text{-CH(OH)-Ph} \\
 & & \text{Configuration} = A
 \end{array}$$

Substrate	R _α	Yield ^a (%)	Ee ^{a,b} (%)
1a	Me	90	>99 (<i>S</i>)
23a	Et	14	97 (<i>S</i>)
25a	<i>i</i> -Pro	4	99 (<i>S</i>)
26a	<i>t</i> -Bu	0	-

The reaction conditions are described in the experimental section. ^a Determined by GC analysis. ^b Higher ee can be obtained when NAD⁺ and 2-propanol are used as a coenzyme and a reducing agent as described above.

It is clear that the substitution of the substrate with electronegative atoms such as fluorine and chlorine inverts the stereochemistry and that this is not because of the bulkiness of the atoms, but it is not clear why the stereochemistry is inverted by an electronic factor. To examine whether the inversion are occurring between the reactions catalyzed by only one enzyme or not, a cell-free extract prepared from APG4 was charged on an anion exchange column and enzymes in APG4 were separated. As shown in Figure 13, when methyl ketone, **1a**, was used as a substrate, several enzymes showed activities of similar strength. The first enzyme eluted at around fraction 29 afforded "A" alcohol whereas the second enzyme eluted around fraction 36 afforded "B" alcohol. The stereoselectivity in the reduction of methyl ketones by the APG4 system is very high and "A" selective even though there are many enzymes with different stereoselectivities present in APG4 because the reduced form of coenzyme (NADPH) is available to only the first eluted enzyme, "A" enzyme, since it can reduce the oxidized form of coenzyme (NADP⁺) by itself using cyclopentanol effectively, whereas the second eluted enzyme, the "B" enzyme, can not. However, when trifluoromethyl ketones were used as a substrate, the activity of the "A" enzyme was negligible compared with that of the "B" enzyme. Therefore, trifluoromethyl ketones are reduced only by the "B" enzyme, and hence the high "B" selectivity. After all, methyl ketones and trifluoromethyl ketones are reduced by enzymes with opposite stereoselectivities.

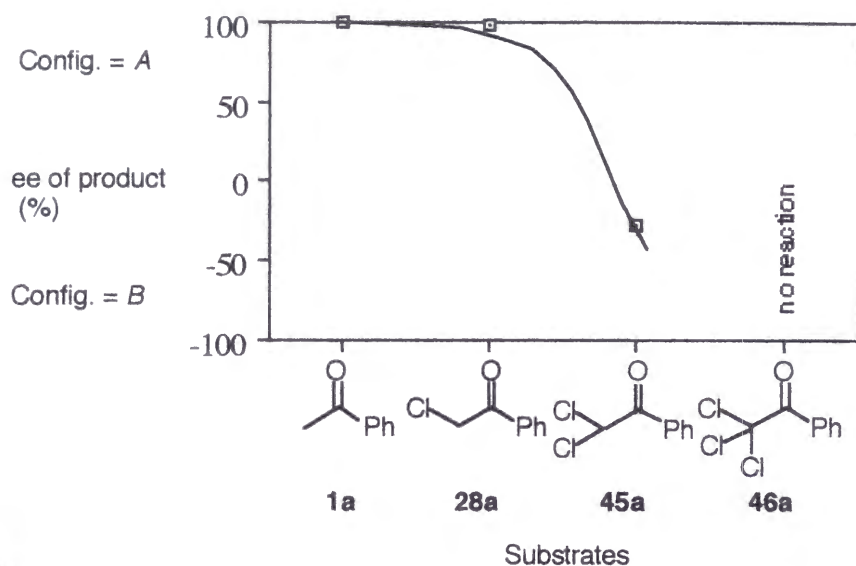


Figure 12. Effect of chlorination at the α -position of acetophenone on the stereoselectivity in the reduction by the APG4 system

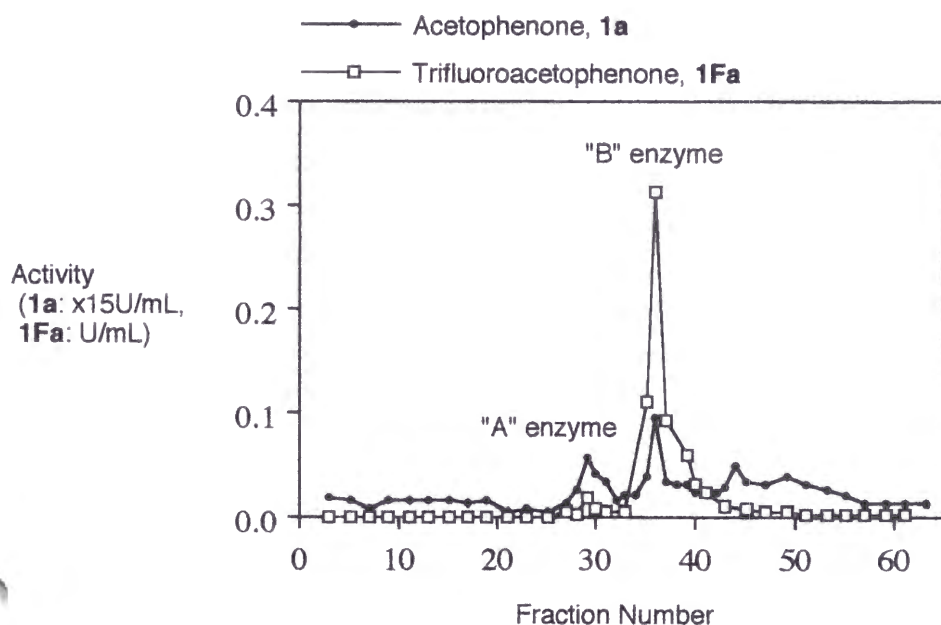


Figure 13. Separation of enzymes in APG4 by anion-exchange column chromatography.

The "B" enzyme reduces the trifluoromethyl ketone about 100 times faster than the methyl ketone, perhaps due to the difference in electrophilicity of the carbonyl carbon rather than the ease of formation of substrate-enzyme complex. The search for a natural substrate of the "B" enzyme is underway in our laboratories. Substrates with fluorine are rare in nature and should shed light on how the fluorine is recognized by the enzyme.

Synthesis of Chiral Halogenated Alcohols

The synthesis of chiral alcohols on a preparative and gram scale is important from practical point of view. The APG4 system and the "B" enzyme were used for the synthesis. For the former, the experimental conditions were examined because both NAD^+ and NADP^+ can be used as a

coenzyme and 2-alkanols from 2-propanol to 2-octanol and cyclopentanol as a reducing agent. The best conditions were chosen and used for the synthesis and the results are listed in Table 12. When the "B" enzyme was used for the reduction, coenzyme was recycled by glucose-6-phosphate and glucose-6-phosphate dehydrogenase, a commonly used recycling system.

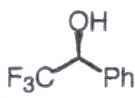
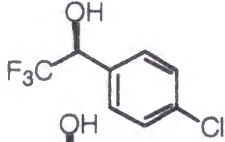
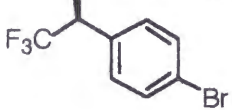
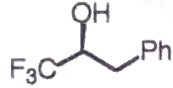
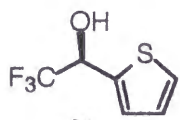
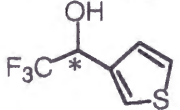
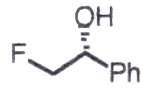
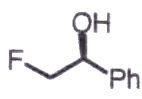
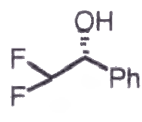
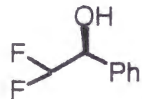
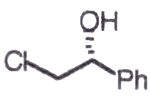
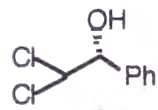
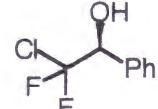
Trifluoromethyl aryl ketones are reduced smoothly and with excellent ee by the APG4 system to the corresponding alcohols, which can be valuable starting materials. For example, the functionality of alcohol and bromine in (*S*)-**10Fb** can be converted and the thienyl group in (*R*)-**41Fb** and (+)-**42Fb** can be used for various reactions³⁷ such as the reduction to aliphatic alcohols, substitution and even polymerization, and its application to electronic material such as the conducting polymer *etc.* would be interesting. Concerning reduction of **41Fa** by the APG4 system, a perfect enantioselectivity and an excellent yield were obtained even when the substrate concentration was increased from 27 mM to 57 mM. The reduction in the gram scale proceeded quantitatively and yielded the optically pure alcohol (*R*)-**41Fb** in 84% yield after purifications (1.32 g, ee >99%).

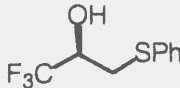
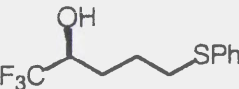
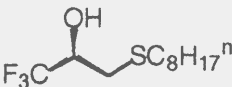
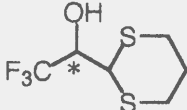
Monofluoroacetophenone (**43a**) and difluoroacetophenone (**44a**) are reduced to (*R*)-alcohols, "A", by the APG4 system when NAD⁺ and 2-propanol are used as the coenzyme and a reducing agent, respectively, and to (*S*)-alcohols, "B", by the B-enzyme. Both enantiomers of monofluorophenylethanol (**43b**) can be obtained with excellent ee, although it is usually difficult to do so using only one kind of microbe.

Both methods can be easily scaled up because the stereoselectivity of the reduction does not depend on the scale and the workup procedure is very simple compared to the whole cell system.

To prepare useful chiral building blocks bearing a trifluoromethyl group, the substrate specificity of the APG4 system has been expanded particularly to trifluoromethyl ketones containing a thioether functionality such as phenylthio and dithianyl groups since they have been used as useful reactive groups for a variety of chemical transformations. Sulfides **48a**, **49a** and **50a** can be reduced successfully. However, a limitation in the substrate specificity of the APG4 system was also found; the reduction of a substrate with dithianyl moiety **51a** did not proceed smoothly. Only when the amount of APG4 was increased, (+)-**51b** was obtained in 42% yield with >99% ee.

Table 12. Synthesis of Chiral Halogenated Alcohols

Product	Catalyst ^a	Coenzyme	Coenzyme Recycle ^b	Yield(%)	Ee ^c (%)
 (<i>S</i>)- 1Fb	APG4	NADP ⁺	Cyclopentanol	84	98 ^d
 (<i>S</i>)- 7Fb	APG4	NADP ⁺	Cyclopentanol	81	>99
 (<i>S</i>)- 10Fb	APG4	NADP ⁺	Cyclopentanol	80	>99
 (<i>S</i>)- 21Fb	APG4	NADP ⁺	Cyclopentanol	74	98
 (<i>R</i>)- 41Fb	APG4	NAD ⁺	Cyclopentanol	84	>99
 (+)- 42Fb	APG4	NAD ⁺	Cyclopentanol	87	>99
 (<i>R</i>)- 43b	APG4	NAD ⁺	2-Propanol	93	>99
 (<i>S</i>)- 43b	B-enz	NADPH	G-6-P	91	>99
 (<i>R</i>)- 44b	APG4	NAD ⁺	2-Propanol	99	63
 (<i>S</i>)- 44b	B-enz	NADPH	G-6-P	95	>99
 (<i>R</i>)- 28b	APG4	NAD ⁺	2-Propanol	49	98
 (<i>R</i>)- 45b	APG4	NAD ⁺	2-Propanol	14	38
 (<i>S</i>)- 47b	APG4	NADP ⁺	Cyclopentanol	82	94

	(<i>R</i>)- 48b	APG4	NAD ⁺	Cyclopentanol	90	98
	(<i>S</i>)- 49b	APG4	NAD ⁺	2-Propanol	78	>99
	(<i>R</i>)- 50b	APG4	NADP ⁺	Cyclopentanol	35 ^f	96
	(+)- 51b	APG4	NAD ⁺	Cyclopentanol	42	>99

The reaction conditions are described in the experimental section. ^a APG4: dried cell of *G. candidum* IFO 4597, B-enz: the B-enzyme separated by anion-exchange chromatography. ^b The indicated alcohol or glucose-6-phosphate dehydrogenase and glucose-6-phosphate (G-6-P) are added to recycle the coenzyme. ^c Determined by GC analysis or HPLC analysis. ^d Ee can be improved to 99% by using a half amount of APG4 and a triple amount of cyclopentanol. ^f The yield is low due to the high volatility (GC yield is 60%).

V. Conclusions

A simple reagent for asymmetric reduction of various kinds of ketones for organic chemists with little background in biochemistry or microbiology has been created. The use of the acetone powder of the microbe eliminates the laborious isolation of the enzyme and problems of low selectivity associated with catalysis by the whole cell. To investigate the substrate specificity, wide variety of ketones was subjected to the APG4 reduction system. The substrate specificity of the acetone powder system is very wide and excellent enantioselectivity of the reduction was obtained for most of the wide variety of substrates. Since the yield of the reduction is also satisfactory, the present method is highly effective for asymmetric synthesis of optically pure aromatic alcohols, aliphatic alcohols, and hydroxy esters.

The stereochemical outcome of the reduction of ketones by the APG4 system depends on the number of halogens at the α -position regardless of the other group adjacent to the carbonyl moiety. The reduction of methyl ketones showed *A*-selectivity, whereas that of trifluoromethyl ketones showed *B*-selectivity. The inversion in stereochemistry is caused not merely by the bulkiness of the trifluoromethyl group but by the electronic factor imparted by the halogens.

The mechanism for the inversion in stereochemistry by the electronegative group at the α -position was investigated by enzymatic studies. It was found that there are several enzymes with different stereoselectivities and even under the exact same conditions, one of the enzymes catalyzes the reduction of methyl ketones, whereas another with the opposite stereoselectivity catalyzes the reduction of trifluoromethyl ketones due to the difference in the activities of the enzymes toward the different kinds of ketones. In other words, the *B*-enzyme reduces fluorinated ketones much more efficiently than the unfluorinated analogues. An investigation of how the halogenated substrates are recognized and bind in the active site of the enzyme would be both interesting and important since the binding of some aromatic pollutants like dioxins to the receptors depends on the chlorination pattern.³⁸

The reduction of fluorinated ketones by the isolated enzyme as well as by APG4 results in the synthesis of chiral alcohols with excellent ee, and both enantiomers of monofluorinated alcohols were obtained. Both the APG4 and the *B*-enzyme system is very convenient for the synthesis of optically pure fluorinated alcohols.

VI. Experimental Section

Instruments. Gas chromatographic analyses were performed using a Shimadzu GC-9A, GC-14A or GC-14B gas chromatograph with a Shimadzu C-R6A Chromatopac equipped with chiral GC-columns (Chiraldex G-TA; 40 m or 30 m; He 2 mL/ min (**G-TA**), CP-Cyclodextrin-B-2,3,6-M-19; 25 m; He 2 mL/ min (**CPCD**), Chirasil-DEX CB; 25 m; He 2 mL/ min (**DEX**)). HPLC analyses were performed using a Hitachi 655 Liquid Chromatograph equipped with Hitachi D-2000 Chromato-Integrator, Spectrophotometer 852 III, and Chiralcel OD (0.46 cm ϕ x 25 cm, hexane : 2-propanol = 5 : 1, 0.5 mL/min (**OD**)). ^1H NMR spectra were recorded at 200 MHz on a Varian VXR-200 spectrometer in CDCl_3 . IR spectra were obtained from JASCO FT/IR-5300 and Shimadzu FT/IR-8100 spectrometers. The optical rotations were measured with 10 cm path-length cells on a JASCO DIP-181 Digital Polarimeter. Elemental analyses were done with a Yanako MT-3 Elemental Analyzer.

Chemicals. Organic reagents were purchased from Nacalai Tesque, Inc., Wako Pure Chemical Industries, Ltd., or Aldrich Chemical Company, Inc. unless noted otherwise. Trifluoroalkanones (**34Fa** - **37Fa**) was kindly supplied by Dr. Ito at Okayama University and trifluoromethyl ketones containing sulfur functionality (**48a** - **51a**) by Prof. Shimizu and Prof. Fujisawa at Mie University.

Cultivation of *Geotrichum candidum*. *Geotrichum candidum* IFO 4597 was grown in a complex medium consisting of 3% (wt/vol) glycerol, 1% (wt/vol) yeast extract, and 0.5% (wt/vl) polypeptone. The medium, adjusted to pH 6.2 using 0.1 M potassium phosphate buffer, was placed in a Sakaguchi flask, sterilized (121 $^{\circ}\text{C}$, 20 min) and inoculated with the preincubated culture. The cultivation was carried out for 24 h at 30 $^{\circ}\text{C}$ with shaking. The cells (18 g wet wt) were obtained by filtration from 1.0 L of the medium.

Preparation of Acetone Powder (APG4). The cells of *Geotrichum candidum* IFO 4597 (18 g wet wt) were mixed with cold acetone ($-20\text{ }^{\circ}\text{C}$) and filtered. The procedure was repeated five times and then dried under reduced pressure. The dried cells (3.8 g) were obtained and used without further purification.

Separation of the Enzymes by Anion-Exchange Column Chromatography. For the separation of the enzymes, APG4 (5.0 g) was homogenized with HEPES buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-NaOH: pH 7.2, 0.1 M, 50 mL), ME (2-mercaptoethanol, 0.04%), DTT (dithiothreitol, 1 mM) and PMSF (α -toluenesulfonyl fluoride, 1 mM) and sonic-disintegrated. The cell-free extract (40 mL) obtained by centrifugation (11,000 rpm, 2 $^{\circ}\text{C}$, 30 min) was added

PMSF (1 mM) and DFP (diisopropyl fluorophosphate: 1 mM) and dialyzed against HEPES (pH 7.2, 10 mM, 750 mL x 2) containing ME (0.04%) and DTT (1 mM). The supernatant (53 mL) obtained by centrifugation (13,000 rpm, 2 °C, 30 min) and filtration through Sterivex-GV (0.22 µm, Millipore) was applied on an anion-exchange column (FPLC system, Pharmacia MonoQ-HR 10/10 column) and eluted with the above buffer and a linear KCl gradient: 0 M for 15 min, 0 M - 0.3 M for 40 min and 0.3 M - 1 M for 10 min. The injection volume was 5 mL, the flow rate was 2 mL/min and the fraction size was 2 mL. The enzyme activity was assayed at 30 °C by measuring the change in absorbance at 340 nm due to the oxidation of NADPH at a substrate concentration of 2.5 mM in Tris-HCl buffer (pH 7.0, 0.1 M). One unit of enzyme oxidizes 1 µmol NADPH to NADP⁺ / min. The B-enzyme eluted around fraction number 36 is collected and used for the preparative scale synthesis.

Whole-Cell (1a). **1a** (0.08 mmol) was added to a suspension of freshly prepared whole cells (0.5 g wet wt) in water (3 mL) and shaken at 130 rpm at 30 °C for 20 h. The resulting mixture was put on Extrelut and eluted with ether. The products were identified by comparing the GC retention times with those of the authentic samples. The chemical yield and ee of 1-phenylethanol (**1b**) were determined by GC analysis of the ether extract to be 52% and 28%, respectively (GC conditions: CPCD, 110 °C).

APG4 (1a). **1a** (0.08 mmol), NAD⁺ (0.007 mol) and 2-propanol (1.31 mmol) were added to a suspension of APG4 (10 mg) in MES (2-(*N*-morpholino)ethanesulfonic acid) buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm at 30 °C for 20 h and the resulting mixture was put on Extrelut and eluted with ether. The products were identified by comparing the GC retention times with those of the authentic samples. Chemical yield and ee of **1b** were determined by GC analysis (GC conditions: CPCD, 110 °C) of the ether extract. A similar experiment was conducted using NADH, NADP⁺, and NADPH as a coenzyme and cyclopentanol as a reducing agent. The results are listed in Table 1.

Ratio of Biocatalyst (APG4) to Substrate (B/S). The reduction was conducted as described in the APG4 section except that the amount of APG4 was changed in order to elucidate its effect on the results. The results are shown in Figure 1.

Coenzyme. The reduction was conducted as described in the APG4 section except that the amount of NAD⁺ was changed in order to elucidate its effect on the results. The results are shown in Figure 2.

Reducing Agents. The reduction was conducted as described in the APG4 section except that

various kinds of alcohols (1-pentanol, 2-pentanol, 3-pentanol, cyclopentanol, cyclohexanol, and ethanol) as reducing agents were used instead of 2-propanol in order to elucidate their effect on the results. The results are shown in Figure 3. The amounts of cyclopentanol, 2-pentanol and 2-propanol as reducing agents were also changed in order to elucidate their concentration effect on the results. The results are shown in Figure 4.

Kinetic Parameters. NAD⁺ (0.007 mol) and 2-propanol (1.31 mmol) were added to APG4 (10 mg) homogenated with MES buffer (pH 7.0, 0.1 M, 1 mL), and the reaction was started by the addition of various concentrations (6 different concentrations) of **1a** solutions of MES buffer (pH 7.0, 0.1 M, 2 mL). The mixture was shaken at 130 rpm at 30 °C. Aliquots were withdrawn from the mixture every 10 min for 1 h, put on Extrelut, extracted with ether and subjected to GC analyses (GC conditions: CPCD, 160 °C). The initial rates of reduction were measured by following the increase in the concentration of the product. The kinetic parameters were determined from the initial rate *via* a Lineweaver-Burk plot to be 1.7 mM and 0.0085 mM•min⁻¹•mg⁻¹ of APG4 for K_m and V_{max}, respectively.

Buffer. **1a** (0.08 mmol), NAD⁺ (0.007 mol) and 2-propanol (1.31 mmol) were added to a suspension of APG4 (10 mg) in various kinds of buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm at 30 °C. Aliquots were withdrawn from the mixture every 20 min for 2 h, put on Extrelut, extracted with ether and subjected to GC analyses (GC conditions: CPCD, 160 °C). The initial rates of reduction were measured by following the increase in the concentration of the product. The reduction rate of acetophenone in MES buffer was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). Chemical yields and ee of **1b** were determined by GC analyses of the ether extract at 20 h (GC conditions: CPCD, 110 °C). The results are shown in Table 2.

pH. The reduction was conducted as described in the Buffer section except that the pH of the buffer was changed in order to elucidate its effect on the results. The results are shown in Figure 5 and Table 3.

Reaction Temperature. The reduction was conducted as described in the APG4 section except that the reaction temperature was changed in order to elucidate its effect on the results, and aliquots were withdrawn from the mixture at various times to see the time course of the reaction. The results are shown in Figure 6.

Thermostability. APG4 (10 mg) was incubated in MES buffer (pH 7.0, 0.1 M, 3 mL) at various temperatures for 30 min, and then **1a** (0.08 mmol), NAD⁺ (0.007 mol) and 2-propanol (1.31 mmol) were added to the suspension of APG4. The mixture was shaken at 130 rpm at 30 °C, and aliquots

were withdrawn from the mixture at appropriate time interval, put on Extrelut, extracted with ether and subjected to GC analyses (GC conditions: CPCD, 160 °C). The initial rates of reduction were measured by following the increase in the concentration of the product. The reduction rate of acetophenone at the standard conditions (see APG4 section) was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). Ee of **1b** were determined by GC analyses of the ether extract at 20 h (GC conditions: CPCD, 110 °C). The results are shown in Figure 7.

Preservability. APG4 was placed in test tubes in an aerobic atmosphere at 30 °C or -20 °C for the indicated periods of time and then **1a** (0.08 mmol), NAD⁺ (0.007 mol), 2-propanol (1.31 mmol) and MES buffer (pH 7.0, 0.1 M, 3 mL) were added to the test tubes. The mixture was shaken at 130 rpm at 30 °C, and aliquots were withdrawn from the mixture every 20 min for 2 h, put on Extrelut, extracted with ether and subjected to GC analyses (GC conditions: CPCD, 160 °C). The initial rates of reduction were measured by following the increase in the concentration of the product. The reduction rate of acetophenone by APG4 freshly prepared was arbitrarily assigned a unit value (the absolute value was 76 nmol/mL/min). Ee of **1b** were determined by GC analyses of the ether extract at 20 h (GC conditions: CPCD, 110 °C). The results are shown in Table 4.

Residual Activity of APG4 after the (1a). **1a** (0.08 mmol), NAD⁺ (0.007 mol) and 2-propanol (1.31 mmol) were added to a suspension of APG4 (10 mg) in MES buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm at 30 °C for 14 h and then additional **1a** (0.08 mmol) was added to the resulting mixture together with: 1) 2-propanol (1.31 mmol) and NAD⁺ (0.007 mmol), 2) NAD⁺ (0.007 mmol), 3) 2-propanol (1.31 mmol), 4) none. At appropriate time periods, aliquots were withdrawn from the mixture, put on Extrelut, extracted with ether and subjected to GC analyses (GC conditions: CPCD, 110 °C or 160 °C) to determine yields and ee. The time course was shown in Figure 8.

Effect of Substituents at the α -Position of Acetophenone on the Enantioselectivity of the APG4 Reduction. A ketone (0.08 mmol), NADP⁺ (0.007 mol) and cyclopentanol (0.65 mmol) were added to a suspension of APG4 (20 mg) in MES buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm and 30 °C for 20 h and the resulting mixture was put on Extrelut and eluted with ether. The chemical yield and ee of the product were determined by GC analysis, HPLC analysis or NMR analysis of the ether extract.

Synthetic Applications.

Preparation of Ketones.

1-Phenyl-2-propanone (21a). This ketone was prepared through the oxidation of 1-phenyl-2-propanol (22 mmol, 3.0 g) by stirring in DMSO (300 mL), DCC (132 mmol, 25 g), and trifluoroacetic acid (33 mmol, 3.8 g) under an atmosphere of argon at room temperature for 4 days. Yield 39% (8.4 mmol, 1.2 g); ^1H NMR (CDCl_3) δ 2.15 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 7.17-7.35 (m, 5H, Ph); IR (neat) 700, 737, 1030, 1078, 1159, 1229, 1358, 1422, 1454, 1497, 1603, 1713, 2926, 3030, 3063 cm^{-1} .

2,2,2-Trifluoro-1-(3-thienyl)ethanone (42Fa): n-BuLi (11 mmol, 1.54 M in hexane solution) was added dropwise to a stirring solution of 3-bromothiophene (10 mmol, 1.63 g) in ether (5 mL) at -18°C . The resulting mixture was added dropwise to a solution of ethyl 2,2,2-trifluoroacetate (10 mmol, 1.42 g) in ether (5 mL) at -70°C , stirred for 10 min at rt, quenched with $\text{NH}_4\text{Cl}(\text{aq})$, extracted with ether, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by distillation with a Kugelrohr apparatus (120°C / 16 mmHg), giving **42Fa** (1.12 g, 62%). ^1H NMR (CDCl_3) δ 7.40-7.45 (m, 1H, Thienyl), 7.66-7.68 (m, 1H, Thienyl), 8.35-8.38 (m, 1H, Thienyl); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -74 (s); IR (neat) 1711 cm^{-1} ; HRMS for ($\text{C}_6\text{H}_3\text{OF}_3\text{S}$) $^+$: Calcd: 179.9857, Found: 179.9839.

α -Fluoroacetophenone (43a): A mixture of α -bromoacetophenone (5 mmol, 1.0 g), 18-crown-6 (0.038 mmol, 10 mg) and anhydrous potassium fluoride (40 mmol, 2.32 g) in acetonitrile (10 mL) was heated under reflux for 24 h, cooled to room temperature, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, hexane : ethyl acetate = 5 : 1) followed by distillation with a Kugelrohr apparatus (170°C / 21 mmHg), giving **43a** (287 mg, 42%). ^1H NMR (CDCl_3) δ 5.53 (d, 2H, CH_2 , $J = 47$ Hz), 7.46-7.63 (m, 3H, Ph), 7.87-7.91 (m, 2H, Ph); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -231 (t, $J = 47$ Hz); IR (neat) 1707 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_7\text{OF}$: C, 69.56%; H, 5.11%. Found: C, 69.33%; H, 5.03%.

α, α -Difluoroacetophenone (44a): **44a** was prepared *via* the Grignard reaction.^{35b} ^1H NMR (CDCl_3) δ 6.30 (t, 1H, $J = 54$ Hz), 7.48-7.57 (m, 2H, Ph), 7.64-7.68 (m, 1H, Ph), 8.05-8.09 (m, 2H, Ph); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -122 (d, $J = 53$ Hz); IR (neat) 1711 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_6\text{OF}_2$: C, 61.54%; H, 3.87%. Found: C, 61.20%; H, 3.82%.

α, α, α -Trichloroacetophenone (46a): **46a** was prepared *via* Friedel-Crafts reaction with AlCl_3 (18 mmol, 2.4 g), trichloroacetyl chloride (19 mmol, 3.4 g) and benzene (32 mL): Yield 96%; ^1H

NMR (CDCl₃) δ 7.46-7.54 (m, 2H, Ph), 7.60-7.68 (m, 1H, Ph), 8.23-8.28 (m, 2H, Ph); IR (neat) 1713 cm⁻¹.

Preparation of racemic alcohols (1b - 51b, 7Fb, 10Fb, 34Fb - 37Fb, 41Fb, 42Fb).

Authentic racemic alcohols (1b - 51b, 7Fb, 10Fb, 34Fb - 37Fb, 41Fb, 42Fb) were prepared by the reduction of the corresponding ketones (1a - 51a, 7Fa, 10Fa, 34Fa - 37Fa, 41Fa, 42Fa, 13 mmol) by NaBH₄ (13 mmol) in EtOH (25 mL) for those which were not commercially available.

General Procedure for the Reduction of Ketones by the APG4 System. A ketone (0.08 mmol), NAD(P)⁺ (5 mg, 0.007 mol) and 2-propanol or cyclopentanol (1.31 mmol) were added to a suspension of APG4 (10 mg) in MES buffer (pH 7.0, 0.1 M, 3 mL). The mixture was shaken at 130 rpm at 30 °C for 20 h, and the resulting mixture was put on Extrelut and eluted with ether. The products were identified by comparing the GC retention times with those of the authentic samples. Chemical yield and ee were determined by GC, HPLC, or NMR analyses of the ether extract. Some alcohols obtained by the APG4 reduction were acetylated to determine ee by GC analyses.

General Procedure for the Reduction of Ketones in Preparative Scale by the APG4 System. A ketone (1.6 mmol), 2-propanol or cyclopentanol (26 mmol, 2.0 mL), NAD(P)⁺ (100 mg) and APG4 (200 mg) were added to 60 ml of MES buffer (0.1 M, pH 7.0). The mixture was stirred at 30 °C for 20 h at 130 rpm under an argon atmosphere and filtered through Extrelut which was washed with ether. The filtrate was extracted with ether and the combined ether solution was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent, hexane : ethyl acetate = 5 : 1 to 3 : 1) followed by distillation with a Kugelrohr apparatus. The product was identified with ¹H NMR, IR, and elemental analysis. Ee was determined by GC analysis or HPLC analysis. An absolute configuration was assigned on the basis of the literature values of optical rotation, unless otherwise noted.

(S)-1-Phenylethanol (1b): 1a (120 mg, 1.0 mmol) was converted to (S)-1b by APG4. Yield 74% (90 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); [α]_D²⁵ -55.1 (*c* 1.63, CHCl₃)(lit.³³ [α]_D²⁵ -57 (*c* 5.12, CHCl₃), *S*); ¹H NMR (CDCl₃) δ 1.50 (d, 3H, CH₃, *J* = 6.6 Hz), 1.83 (br s, 1H, OH), 4.90 (q, 1H, CH, *J* = 6.5 Hz), 7.24-7.40 (m, 5H, Ph); IR (neat) 700, 760, 899, 1011, 1078, 1204, 1370, 1453, 2975, 3356 cm⁻¹; Anal. Calcd. for C₈H₁₀O: C, 78.65%; H, 8.25%. Found: C, 78.43%; H, 8.28%.

(S)-1-*o*-Fluorophenylethanol (2b): 2a (1.11 g, 8.0 mmol) was converted to (S)-2b by APG4.

Yield 94% (1.06 g); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{25}$ -44.5 (c 0.782, MeOH). The absolute configuration of this compound was tentatively assigned to be *S*. ^1H NMR (CDCl_3) δ 1.50 (d, 3H, CH_3 , J = 6.6), 2.04 (br s, 1H, OH), 5.19 (q, 1H, CH, J = 6.1), 6.95 - 7.10 (m, 1H, Ph), 7.13 - 7.29 (m, 2H, Ph), 7.43 - 7.52 (m, 1H, Ph); IR (neat) 696, 787, 872, 936, 1144, 1267, 1451, 1593, 2976, 3356 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OF}$: C, 68.56%; H, 6.47%. Found: C, 68.31%; H, 6.58%.

(*S*)-1-*m*-Fluorophenylethanol (3b): **3a** (1.11 g, 8.0 mmol) was converted to (*S*)-**3b** by APG4. Yield 90% (1.01 g); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{25}$ -33.5 (c 1.435, MeOH). The absolute configuration of this compound was tentatively assigned to be *S*. ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , J = 6.4), 1.89 (br s, 1H, OH), 4.89 (q, 1H, CH, J = 6.7), 6.89 - 7.00 (m, 1H, Ph), 7.03 - 7.14 (m, 2H, Ph), 7.25 - 7.35 (m, 1H, Ph); IR (neat) 758, 824, 1011, 1078, 1225, 1453, 1489, 2978, 3356 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OF}$: C, 68.56%; H, 6.47%. Found: C, 68.37%; H, 6.36%.

(*S*)-1-*p*-Fluorophenylethanol (4b): **4a** (360 mg, 2.6 mmol) was converted to (*S*)-**4b** by APG4. Yield 60% (219 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 115 °C); $[\alpha]_D^{25}$ -37.7 (c 0.931, MeOH)(lit.³⁹ $[\alpha]_D^{25}$ -23.1 (c 3.12, MeOH), 70% ee(*S*)); ^1H NMR (CDCl_3) δ 1.47 (d, 3H, CH_3 , J = 6.4), 1.80 (br s, 1H, OH), 4.89 (q, 1H, OH, J = 6.6), 6.98 - 7.08 (m, 2H, Ph), 7.30 - 7.37 (m, 2H, Ph); IR (neat) 899, 1013, 1084, 1157, 1225, 1510, 1605, 2976, 3362 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OF}$: C, 68.56%; H, 6.47%. Found: C, 68.34%; H, 6.44%.

(*S*)-1-*o*-Chlorophenylethanol (5b): **5a** (365 mg, 2.4 mmol) was converted to (*S*)-**5b** by APG4. Yield 94% (346 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 130 °C); $[\alpha]_D^{25}$ -62.7 (c 0.894, CHCl_3)(lit.^{29e} $[\alpha]_D^{25}$ -56.5 (c 0.0463, CHCl_3) 90% ee(*S*)); ^1H NMR (CDCl_3) δ 1.47 (d, 3H, CH_3 , J = 6.4), 2.26 (s, 1H, OH), 5.27 (q, 1H, CH, J = 6.4), 7.14 - 7.29 (m, 3H, Ph), 7.55 - 7.60 (m, 1H, Ph); IR (neat) 754, 901, 1009, 1038, 1047, 1094, 1439, 1474, 2976, 3349 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OCl}$: C, 61.35%; H, 5.79%. Found: C, 61.17%; H, 5.96%.

(*S*)-1-*m*-Chlorophenylethanol (6b): **6a** (154 mg, 1.0 mmol) was converted to (*S*)-**6b** by APG4. Yield 91% (143 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 130 °C); $[\alpha]_D^{25}$ -43.5 (c 1.08, CHCl_3)(lit.⁴⁰ $[\alpha]_D^{20}$ +36.7 (c 1.0, CHCl_3) 84.6% ee(*R*)); ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , J = 6.4 Hz), 1.81 (br s, 1H, OH), 4.88 (q, 1H, CH, J = 6.4 Hz),

7.22-7.27 (m, 3H, Ph), 7.36-7.37 (m, 1H, Ph); IR (neat) 696, 787, 1078, 1202, 1431, 1574, 1599, 2976, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OCl}$: C, 61.35%; H, 5.79%. Found: C, 61.29%; H, 5.86%.

(S)-1-*p*-Chlorophenylethanol (7b): **7a** (362 mg, 2.3 mmol) was converted to (*S*)-**7b** by APG4. Yield 80% (293 mg); ee >99% (Determined by GC analysis. GC conditions: G-TA, 135 °C); $[\alpha]_D^{25}$ -49.0 (*c* 1.84, ether)(lit.^{29e} $[\alpha]_D$ -48.9 (*c* 0.0613, ether) 94% ee(*S*)); ^1H NMR (CDCl_3) δ 1.45 (d, 3H, CH_3 , $J = 6.4$), 2.09 (s, 1H, OH), 4.85 (q, 1H, CH, $J = 6.5$), 7.29 (s, 4H, Ph); IR (neat) 829, 899, 1013, 1090, 1493, 2975, 3351 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OCl}$: C, 61.35%; H, 5.79%. Found: C, 61.24%; H, 5.78%.

(S)-1-*o*-Bromophenylethanol (8b): **8a** (2.6 g, 13 mmol) was converted to (*S*)-**8b** by APG4. Yield 89% (2.3 g); ee >99% (Determined by GC analysis. GC conditions: CPCD, 145 °C); $[\alpha]_D^{24}$ -54.6 (*c* 1.23, CHCl_3)(lit.^{29e} $[\alpha]_D$ -50.5 (*c* 0.0305, CHCl_3) 94% ee(*S*)); ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , $J = 6.2$ Hz), 2.03 (br s, 1H, OH), 5.23 (q, 1H, CH, $J = 6.2$ Hz), 7.08-7.16 (m, 1H, Ph), 7.26-7.37 (m, 1H, Ph), 7.48-7.61 (m, 2H, Ph); IR (neat) 754, 899, 1024, 1092, 1441, 1468, 2975, 3349 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OBr}$: C, 47.79%; H, 4.51%. Found: C, 47.85%; H, 4.55%.

(S)-1-*m*-Bromophenylethanol (9b): **9a** (1.00 g, 5.0 mmol) was converted to (*S*)-**9b** by APG4. Yield 85% (854 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 145 °C); $[\alpha]_D^{26}$ -28.6 (*c* 1.78, EtOH); The absolute configuration was determined to be *S* by debromination of **9b** to (*S*)-1-phenylethanol as explained in the scheme 2. ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , $J = 6.2$ Hz), 1.84 (d, 1H, OH, $J = 3.2$ Hz), 4.81-4.91 (m, 1H, CH), 7.17-7.54 (m, 4H, Ph); IR (neat) 696, 783, 907, 1013, 1071, 1200, 1427, 1570, 1595, 2975, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OBr}$: C, 47.79%; H, 4.51%. Found: C, 47.66%; H, 4.50%.

(S)-1-*p*-Bromophenylethanol (10b): **10a** (100 mg, 0.50 mmol) was converted to (*S*)-**10b** by APG4. Yield 66% (67 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 150 °C); $[\alpha]_D^{23}$ -37.9 (*c* 1.13, CHCl_3)(lit.^{29e} $[\alpha]_D$ -37.5 (*c* 0.0666 CHCl_3) 96% ee(*S*)); ^1H NMR (CDCl_3) δ 1.46 (d, 3H, CH_3 , $J = 6.2$ Hz), 1.9 (br s, 1H, OH), 4.86 (q, 1H, CH, $J = 6.2$ Hz), 7.20-7.27 (m, 2H, Ph), 7.42-7.50 (m, 2H, Ph); IR (neat) 824, 898, 1009, 1086, 1402, 1489, 2975, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_9\text{OBr}$: C, 47.79%; H, 4.51%. Found: C, 48.07%; H, 4.60%.

(S)-1-*o*-Methylphenylethanol (11b): **11a** (362 mg, 2.7 mmol) was converted to (*S*)-**11b** by

APG4. Yield 73% (269 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 130 °C); $[\alpha]_D^{25}$ -64.3 (*c* 1.04, EtOH)(lit.^{29e} $[\alpha]_D$ -58.6 (*c* 0.0665, EtOH) 95% ee(*S*)); ¹H NMR (CDCl₃) δ 1.46 (d, 3H, CH₃, *J* = 6.4), 1.96 (br s, 1H, OH), 2.34 (s, 3H, *o*-CH₃), 5.11 (q, 1H, CH, *J* = 6.4), 7.11-7.27 (m, 3H, Ph), 7.49-7.53 (m, 1H, Ph); IR (neat) 727, 760, 897, 1005, 1076, 1370, 1460, 2973, 3356 cm⁻¹; Anal. Calcd. for C₉H₁₂O: C, 79.37%; H, 8.88%. Found: C, 79.43%; H, 8.94%.

(*S*)-1-*m*-Methylphenylethanol (12b): **12a** (309 mg, 2.3 mmol) was converted to (*S*)-**12b** by APG4. Yield 83% (261 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{25}$ -39.8 (*c* 0.944, EtOH)(lit.⁴¹ $[\alpha]_D^{25}$ -41.9 (*c* 0.500, EtOH) >99.9% ee(*S*)); ¹H NMR (CDCl₃) δ 1.48 (d, 3H, CH₃, *J* = 6.6), 1.96 (br s, 1H, OH), 2.36 (s, 3H, *m*-CH₃), 4.85 (q, 1H, CH, *J* = 6.4), 7.07 - 7.28 (m, 4H, Ph); IR (neat) 704, 787, 922, 1078, 1161, 1370, 1451, 2975, 3358 cm⁻¹; Anal. Calcd. for C₉H₁₂O: C, 79.37%; H, 8.88%. Found: C, 79.28%; H, 8.90%.

(*S*)-1-*p*-Methylphenylethanol (13b): **13a** (311 mg, 2.3 mmol) was converted to (*S*)-**13b** by APG4. Yield 70% (220 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{26}$ -43.5 (*c* 0.994, MeOH)(lit.³⁹ $[\alpha]_D^{27}$ -22.3 (*c* 3.79, MeOH) 55% ee(*S*)); ¹H NMR (CDCl₃) δ 1.48 (d, 3H, CH₃, *J* = 6.2), 1.83 (br s, 1H, OH), 2.34 (s, 3H, *p*-Me), 4.86 (q, 1H, CH, *J* = 6.5), 7.14 - 7.18 (d, 2H, Ph, *J* = 8.0), 7.24 - 7.28 (d, 2H, Ph, *J* = 8.2); IR (neat) 818, 899, 1011, 1088, 1370, 1451, 1514, 2973, 3362 cm⁻¹; Anal. Calcd. for C₉H₁₂O: C, 79.37%; H, 8.88%. Found: C, 79.44%; H, 8.77%.

(*S*)-1-*o*-Methoxyphenylethanol (14b): **14a** (557 mg, 3.7 mmol) was converted to (*S*)-**14b** by APG4. Yield 85% (477 mg); ee >99% (*S*) (Determined by GC analysis. GC conditions: CPCD, 125 °C); $[\alpha]_D^{23}$ -63.0 (*c* 1.10, toluene)(lit.⁴⁰ $[\alpha]_D^{20}$ +48.9 (*c* 1.1, toluene) 81.5% ee(*R*)); ¹H NMR (CDCl₃) δ 1.50 (d, 3H, CH₃, *J* = 6.4 Hz), 2.75 (d, 1H, OH, *J* = 3.2 Hz), 3.85 (s, 3H, CH₃O), 5.04-5.14 (m, 1H, CH), 6.85-6.99 (m, 2H, Ph), 7.20-7.37 (m, 2H, Ph); IR (neat) 754, 802, 899, 1030, 1078, 1240, 1464, 1491, 1601, 2971, 3395 cm⁻¹; Anal. calcd. for C₉H₁₂O₂: C, 71.02%; H, 7.95%. Found: C, 70.75%; H, 8.01%.

(*S*)-1-*m*-Methoxyphenylethanol (15b): **15a** (352 mg, 2.3 mmol) was converted to (*S*)-**15b** by APG4. Yield 83% (296 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 130 °C); $[\alpha]_D^{22}$ -34.9 (*c* 0.849, MeOH)(lit.⁴² $[\alpha]_D$ +35 (*c* 1, MeOH) 97% ee(*R*)); ¹H NMR (CDCl₃) δ 1.48 (d, 3H, CH₃, *J* = 6.2), 1.88 (br s, 1H, OH), 3.81 (s, 3H, CH₃O), 4.87 (q, 1H, CH, *J* = 6.2),

6.78 - 6.84 (m, 1H, Ph), 6.92 - 6.95 (m, 2H, Ph), 7.22 - 7.30 (m, 1H, Ph); IR (neat) 700, 785, 856, 1046, 1073, 1159, 1260, 1487, 1603, 2973, 3382 cm^{-1} ; Anal. calcd. for $\text{C}_9\text{H}_{12}\text{O}_2$: C, 71.02%; H, 7.95%. Found: C, 70.72%; H, 8.00%.

(S)-1-*p*-Methoxyphenylethanol (16b): **16a** (704 mg, 4.7 mmol) was converted to (S)-**16b** by APG4. Yield 21% (152 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 130 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{22}$ -51.9 (*c* 0.718, CHCl_3)(lit.^{29e} $[\alpha]_{\text{D}}^{22}$ -46.2 (*c* 0.0273, CHCl_3) 87% ee(S)); ^1H NMR (CDCl_3) δ 1.47 (d, 3H, CH_3 , J = 6.6), 1.80 (br s, 1H, OH), 3.80 (s, 3H, CH_3O), 4.85 (q, 1H, CH, J = 6.4), 6.85 - 6.91 (m, 2H, Ph), 7.25 - 7.33 (m, 2H, Ph); IR (neat) 833, 897, 1036, 1088, 1177, 1246, 1302, 1512, 1613, 2971, 3382 cm^{-1} ; Anal. Calcd. for $\text{C}_9\text{H}_{12}\text{O}_2$: C, 71.02%; H, 7.95%. Found: C, 70.85%; H, 8.09%.

(S)-1-*o*-Trifluoromethylphenylethanol (17b): **17a** (2.0 g, 11 mmol) was converted to (S)-**17b** by APG4. Yield 13% (257 mg); ee 99% (Determined by GC analysis. GC conditions: CPCD, 110 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{22}$ -45.5 (*c* 0.661, MeOH)(lit.³⁹ $[\alpha]_{\text{D}}^{22}$ -23.0 (*c* 1.26, MeOH) 55% ee(S)); ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , J = 6.4), 1.97 (br s, 1H, OH), 5.32 (q, 1H, CH, J = 5.4), 7.32 - 7.39 (m, 1H, Ph), 7.55 - 7.62 (m, 2H, Ph), 7.80 - 7.84 (m, 1H, Ph); IR (neat) 658, 770, 901, 1038, 1059, 1090, 1123, 1165, 1269, 1314, 1456, 1609, 2984, 3356 cm^{-1} ; Anal. Calcd. for $\text{C}_9\text{H}_9\text{OF}_3$: C, 56.84%; H, 4.77%. Found: C, 56.63%; H, 4.69%.

(S)-1-*m*-Trifluoromethylphenylethanol (18b): **18a** (350 mg, 1.9 mmol) was converted to (S)-**18b**. Yield 83% (294 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 120 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{22}$ -28.4 (*c* 1.26, MeOH)(lit.³⁹ $[\alpha]_{\text{D}}^{24}$ -17.1 (*c* 2.92, MeOH) 59% ee(S)); ^1H NMR (CDCl_3) δ 1.51 (d, 3H, CH_3 , J = 6.4), 1.94 (br s, 1H, OH), 4.96 (q, 1H, CH, J = 6.4), 7.25 - 7.64 (m, 4H Ph); IR (neat) 704, 804, 910, 1015, 1073, 1127, 1165, 1271, 1331, 1453, 2978, 3349 cm^{-1} ; Anal. calcd. for $\text{C}_9\text{H}_9\text{OF}_3$: C, 56.84%; H, 4.77%. Found: C, 56.62%; H, 4.70%.

(S)-1-*p*-Trifluoromethylphenylethanol (19b): **19a** (350 mg, 1.9 mmol) was converted to (S)-**19b**. Yield 85% (299 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 120 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{22}$ -28.1 (*c* 1.13, MeOH)(lit.^{29e} $[\alpha]_{\text{D}}^{21}$ -10.8 (*c* 0.0578, MeOH) 61% ee(S)); ^1H NMR (CDCl_3) δ 1.50 (d, 3H, CH_3 , J = 6.4), 1.89 (br s, 1H, OH), 4.97 (q, 1H, CH, J = 6.0), 7.48 (d, 2H, Ph, J = 7.8), 7.60 (d, 2H, Ph, J = 8.4); IR (neat) 739, 843, 901, 1017, 1069, 1090, 1127, 1165, 1329, 1416, 1622, 2978, 3356 cm^{-1} ; Anal. calcd. for $\text{C}_9\text{H}_9\text{OF}_3$: C, 56.84%; H, 4.77%. Found: C, 56.72%; H, 4.70%.

(S)-1-(1',2',3',4',5'-pentafluorophenyl)ethanol (20b): **20a** (281 mg, 1.3 mmol) was converted to **(S)-20b** by APG4. Yield 80% (227 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 120 °C); $[\alpha]_D^{22}$ -6.33 (*c* 1.01, pentane)(lit.⁴³ $[\alpha]_D$ -8.1, (pentane) 97% ee(*S*)); ¹H NMR (CDCl₃) δ 1.64 (d, 3H, CH₃, *J* = 6.6 Hz), 2.16 (d, 1H, OH, *J* = 7.8 Hz), 5.25 (m, 1H, CH); IR (neat) 866, 974, 1046, 1086, 1134, 1304, 1505, 1524, 1653, 2990, 3358 cm⁻¹; Anal. Calcd. for C₈H₅OF₅: C, 45.30%; H, 2.38%. Found: C, 45.39%; H, 2.34%.

(S)-1-Phenyl-2-propanol (21b): **21a** (367 mg, 2.7 mmol) was converted to **(S)-21b** by APG4. Yield 78% (291 mg); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{25}$ +41.7 (*c* 1.19, CHCl₃)(lit.⁴¹ $[\alpha]_D^{25}$ +39.7 (*c* 0.515, CHCl₃) >99.9% ee(*S*)); ¹H NMR (CDCl₃) δ 1.24(d, 3H, CH₃, *J* = 6.2), 1.58 (br s, 1H, OH), 2.69 (dd, 1H, *J* = 13.4, 7.8 Hz), 2.79 (dd, 1H, *J* = 13.5, 4.9 Hz) 3.97-4.07 (m, 1H, CH), 7.18-7.36 (m, 5H, Ph); IR (neat) 700, 743, 941, 1080, 1119, 1373, 1454, 1497, 2930, 2971, 3029, 3374 cm⁻¹; Anal. Calcd. for C₉H₁₂O: C, 79.37%; H, 8.88%. Found: C, 79.25%; H, 8.93%.

(S)-4-Phenyl-2-butanol (22b): **22a** (350 mg, 2.4 mmol) was converted to **(S)-22b** by APG4. Yield 95% (338 mg); ee 98% (Determined by GC analysis. GC conditions: CPCD, 105 °C); $[\alpha]_D^{25}$ +21.0 (*c* 1.17, C₆H₆)(lit.⁴¹ $[\alpha]_D^{22}$ +22 (*c* 0.380, C₆H₆) >99.9% ee(*S*)); ¹H NMR (CDCl₃) δ 1.23 (d, 3H, CH₃, *J* = 6.2 Hz), 1.50 (br s, 1H, OH), 1.71-1.83 (m, 2H, CH₂), 2.59-2.81 (m, 2H, CH₂), 3.75-3.90 (m, 1H, CH), 7.15-7.33 (m, 5H, Ph); IR (neat) 698, 747, 955, 1055, 1128, 1373, 1454, 1495, 2928, 2967, 3027, 3356 cm⁻¹; Anal. Calcd. for C₁₀H₁₄O: C, 79.95%; H, 9.39%. Found: C, 79.83%; H, 9.58%.

(S)-1-Phenyl-1-propanol (23b): **23a** (345 mg, 2.6 mmol) was converted to **(S)-23b** by APG4. Yield 25% (86 mg); ee >99% (Determined by GC analysis. GC conditions: CPCD, 110 °C); $[\alpha]_D^{25}$ -47.2 (*c* 0.643, CHCl₃)(lit.^{29e} $[\alpha]_D$ -46.7 (*c* 0.0409, CHCl₃, 95% ee(*S*)); ¹H NMR (CDCl₃) δ 0.91 (t, 3H, CH₃, *J* = 7.4 Hz), 1.56 (s, 1H, OH), 1.70-1.87 (m, 2H, CH₂), 4.60 (t, 1H, CH, *J* = 6.6), 7.23-7.36 (m, 5H, Ph); IR (neat) 700, 762, 974, 1013, 1096, 1202, 1454, 1493, 2876, 2934, 2967, 3374 cm⁻¹; Anal. Calcd. for C₉H₁₂O: C, 79.37%; H, 8.88%. Found: C, 79.09%; H, 8.79%.

(S)-2-Methyl-1-phenyl-1-propanol (25b): **25a** (1.00 g, 6.7 mmol) was converted to **(S)-25b** by APG4. Yield 11% (113 mg); ee >99% (Determined by GC analysis. GC conditions: G-TA, 95 °C); $[\alpha]_D^{26}$ -49.1 (*c* 0.828, ether)(lit.^{29e} $[\alpha]_D$ -45.7 (*c* 0.0623, ether) 92% ee(*S*)); ¹H NMR CDCl₃ δ

0.79 (d, 3H, CH₃, $J = 6.8$ Hz), 1.00 (d, 3H, CH₃, $J = 6.6$ Hz), 1.82 (d, 1H, CH, $J = 3.0$ Hz), 1.96 (m, 1H, CH, $J = 6.7$ Hz), 4.36 (dd, 1H, CH, $J = 2.8, 7.0$ Hz) and 7.25-7.34 (m, 5H, Ph); IR (neat) 702, 760, 1022, 1383, 1454, 1493, 2961, 3030, 3387 cm⁻¹; Anal. Calcd. for C₁₀H₁₄O: C, 79.95; H, 9.39%. Found: C, 79.80%; H, 9.57%.

(R)-2-Methoxy-1-phenylethanol (27b): **27a** (2.0 g, 13 mmol) was converted to **(R)-27b** by APG4. Yield 5% (99 mg); ee >99% (Determined by GC analysis. GC conditions: DEX, 120 °C); $[\alpha]_D^{25}$ -40.2 (c 1.02, acetone)(lit.⁴⁴ $[\alpha]_D$ -34.4 (c 2, acetone) 76 ee(*R*)); ¹H NMR CDCl₃ δ 3.33-3.58 (m, 2H, CH₂), 3.43 (s, 3H, OCH₃), 4.89 (dd, 1H, CH, $J = 3.3, 8.7$ Hz), 7.25-7.40 (m, 5H, Ph); IR (neat) 700, 758, 905, 970, 1067, 1121, 1196, 1454, 2892, 3441 cm⁻¹; Anal. Calcd. for C₉H₁₂O₂: C, 71.03; H, 7.95%. Found: C, 71.29%; H, 7.96%.

(R)-2-Chloro-1-Phenylethanol (28b): **28a** (1.01 g, 6.5 mmol) was converted to **(R)-28b** by APG4. Yield 49% (493 mg); ee 97% (Determined by HPLC analysis. HPLC conditions: OD); $[\alpha]_D^{25}$ -50.4 (c 1.78, cyclohexane)(lit.⁴⁵ $[\alpha]_D^{25}$ -48.1 (c 1.73, cyclohexane) 100% ee (*R*)); ¹H NMR (CDCl₃) δ 2.67 (m, 1H, OH), 3.64 (dd, 1H, CH₂Cl, $J = 11.3, 8.6$ Hz), 3.74 (dd, 1H, CH₂Cl, $J = 11.3, 3.6$ Hz), 4.86-4.94 (m, 1H, CH), 7.29-7.45 (m, 5H, Ph); IR (neat) 613, 698, 723, 770, 1065, 1200, 1454, 1495, 3032, 3405 cm⁻¹; Anal. Calcd for C₈H₉OCl: C, 61.35%; H, 5.79%. Found: C, 61.21%; H, 5.82%.

(S)-Ethyl 3-hydroxybutanoate (30b): **30a** (136 mg, 1.0 mmol) was converted to **(S)-30b** by APG4. Yield 59% (78 mg, GC Yield >99%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: G-TA, 105 °C); $[\alpha]_D^{25}$ +40.6 (c 1.09, CHCl₃)(lit.⁴⁶ $[\alpha]_D^{25}$ +39.9 (c 1.8, CHCl₃) 92% ee(*S*)); ¹H NMR (CDCl₃) δ 1.22-1.32 (m, 6H, CH₃ and CH₂CH₃), 2.36-2.58 (m, 2H, CH₂), 3.08 (br s, 1H, OH), 4.12 - 4.22 (m, 3H, CH and CH₂CH₃); IR (neat) 949, 1030, 1092, 1182, 1300, 1375, 1736, 2978, 3451 cm⁻¹; Anal. Calcd. for C₆H₁₂O₃: C, 54.53%; H, 9.15%. Found: C, 54.38%; H, 9.19%.

(S)-2,2-Dimethylpropyl 3-hydroxybutanoate (32b): **32a** (172 mg, 1.0 mmol) was converted to **(S)-32b** by APG4. Yield 74% (128 mg, GC Yield >99%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: DEX, 115 °C); $[\alpha]_D^{25}$ +31.1 (c 1.01, CHCl₃)(lit.⁴⁷ $[\alpha]_D^{25}$ +26 (c 1.0, CHCl₃) 80% ee(*S*)); ¹H NMR (CDCl₃) δ 0.95 (s, 9H, CH₃), 1.23 (d, 3H, CH₃, $J = 6.4$ Hz), 2.47-2.52 (m, 2H, CH₂), 3.07 (br s, 1H, OH), 3.82 (s, 2H, OCH₂), 4.12 - 4.30 (m, 1H, CH); IR (neat) 947, 1005, 1086, 1123, 1177, 1294, 1377, 1736,

2963, 3437 cm^{-1} ; Anal. Calcd. for $\text{C}_9\text{H}_{18}\text{O}_3$: C, 62.04%; H, 10.41%. Found: C, 61.82%; H, 10.53%.

(S)-Ethyl 3-hydroxypentanoate (33b): **33a** (500 mg, 3.5 mmol) was converted to (S)-**33b** by APG4. Yield 52% (262 mg, GC Yield 67%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: G-TA, 110 $^{\circ}\text{C}$); $[\alpha]_D^{26} +34.3$ (c 1.05, CHCl_3)(lit.⁴⁶ $[\alpha]_D^{25} +32.2$ (c 5.1, CHCl_3) 93% ee(S)); ^1H NMR (CDCl_3) δ 0.95 (t, 3H, CH_3 , $J = 7.3$ Hz), 1.27 (t, 3H, CH_3 , $J = 7.1$ Hz), 1.44 - 1.58 (m, 2H, CH_2), 2.39 (dd, 1H, CH_2 , $J = 8.8, 16.4$ Hz), 2.50 (dd, 1H, CH_2 , $J = 3.6, 16.4$ Hz), 2.93 (br s, 1H, OH), 3.86 - 3.97 (m, 1H, CH), 4.17 (q, 2H, CH_2 , $J = 7.1$ Hz); IR (neat) 984, 1034, 1111, 1179, 1283, 1375, 1464, 1734, 2936, 2971, 3445 cm^{-1} ; Anal. Calcd. for $\text{C}_7\text{H}_{14}\text{O}_3$: C, 57.51%; H, 9.65%. Found: C, 57.27%; H, 9.66%.

(S)-2-Octanol (34b): **34a** (402 mg, 3.1 mmol) was converted to (S)-**34b** by APG4. Yield 62% (252 mg, GC Yield 89%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: CPCD, 100 $^{\circ}\text{C}$); $[\alpha]_D^{25} +9.00$ (c 1.23, CHCl_3)(lit.^{9a} $[\alpha]_D +8.78$ (CHCl_3) 97% ee(S)); ^1H NMR (CDCl_3) δ 0.87 (t, 3H, CH_3 , $J = 6.4$ Hz), 1.17 (d, 3H, CH_3 , $J = 6.2$), 1.27-1.47 (m, 10H, CH_2), 1.61 (s, 1H, OH), 3.71-3.86 (m, 1H, CH); IR (neat) 939, 1069, 1115, 1375, 1464, 2859, 2928, 2961, 3347 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_{18}\text{O}$: C, 73.78%; H, 13.92%. Found: C, 73.92%; H, 14.16%.

(S)-2-Nonanol (35b): **35a** (399 mg, 2.8 mmol) was converted to (S)-**35b** by APG4. Yield 77% (313 mg, GC Yield 90%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: CPCD, 110 $^{\circ}\text{C}$); $[\alpha]_D^{25} +8.66$ (c 1.18, CHCl_3)(lit.^{9a} $[\alpha]_D +7.96$ (CHCl_3) 98% ee(S)); ^1H NMR (CDCl_3) δ 0.87 (t, 3H, CH_3 , $J = 6.5$ Hz), 1.17 (d, 3H, CH_3 , $J = 6.2$), 1.22-1.47 (m, 12H, CH_2), 1.65 (s, 1H, OH), 3.70-3.86 (m, 1H, CH); IR (neat) 723, 930, 1076, 1115, 1375, 1464, 2857, 2928, 2961, 3349 cm^{-1} ; Anal. Calcd. for $\text{C}_9\text{H}_{20}\text{O}$: C, 74.93%; H, 13.98%. Found: C, 74.64%; H, 14.19%.

(S)-2-Decanol (36b): **36a** (209 mg, 1.3 mmol) was converted to (S)-**36b** by APG4. Yield 49% (103 mg, GC Yield 82%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: CPCD, 125 $^{\circ}\text{C}$); $[\alpha]_D^{23} +10.3$ (c 0.80, EtOH)(lit.⁴⁸ $[\alpha]_D^{25} +4.22$ (c 6.28, EtOH) 83.0% ee(S)); ^1H NMR CDCl_3 δ 0.87 (t, 3H, CH_3 , $J = 6.2$ Hz), 1.17 (d, 3H, CH_3 , $J = 6.2$ Hz) 1.26-1.47 (m, 15H, CH_2 and OH) and 3.74-3.83 (m, 1H, CH); IR (neat) 721, 943, 1082,

1115, 1306, 1375, 1464, 2857, 2926, 2961, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_{10}\text{H}_{22}\text{O}$: C, 75.88; H, 14.01%. Found: C, 75.83%; H, 13.98%.

(S)-2-Undecanol (37b): **37a** (450 mg, 2.6 mmol) was converted to (S)-**37b** by APG4. Yield 61% (276 mg, GC Yield 90%); ee >99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: CPCD, 130 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{21} +7.92$ (c 0.75, EtOH). The absolute configuration of this compound was determined to be *S* by comparison of the optical rotation with that of the authentic sample synthesized as in the scheme 2. ^1H NMR (CDCl_3) δ 0.87 (t, 3H, CH_3 , $J = 6.4$ Hz), 1.17 (d, 3H, CH_3 , $J = 6.2$ Hz), 1.22-1.40 (m, 17H), 3.73-3.79 (m, 1H); IR (neat) 721, 849, 937, 1117, 1375, 1466, 2855, 2926, 2961, 3345 cm^{-1} ; Anal. Calcd. for $\text{C}_{11}\text{H}_{24}\text{O}$: C, 76.67%; H, 14.04%. Found: C, 76.52%; H, 14.03%.

(S)-Sulcatol (38b): **38a** (397 mg, 3.2 mmol) was converted to (S)-**38b** by APG4. Yield 76% (306 mg, GC Yield 92%), ee >99% (Determined by GC analysis. GC conditions: G-TA, 85 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{25} +12.0$ (c 2.93, CHCl_3)(lit.^{9a} $[\alpha]_{\text{D}} +10.76$ (CHCl_3) 99% ee(*S*)); ^1H NMR (CDCl_3) δ 1.18(d, 3H, CH_3 , $J = 6.2$ Hz), 1.42-1.52 (m, 3H, CH_2 and OH), 1.61 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.06 (q, 2H, CH_2 , $J = 7.3$ Hz), 3.79 (m, 1H, CH), 5.12 (t, 1H, CH, $J = 7.2$); IR (neat) 953, 1074, 1128, 1377, 1451, 2926, 2969, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_{16}\text{O}$: C, 74.94%; H, 12.58%. Found: C, 74.92%; H, 12.83%.

(S)-5-Chloro-2-pentanol (39b): **39a** (612 mg, 5.1 mmol) was converted to (S)-**39b** by APG4. Yield 40% (249 mg, GC Yield 93%), ee 99% (The product alcohol was acetylated to determined ee by GC analysis. GC conditions: DEX, 110 $^{\circ}\text{C}$); $[\alpha]_{\text{D}}^{26} +14.9$ (c 1.19, CHCl_3)(lit.^{9a} $[\alpha]_{\text{D}} +15.58$ (CHCl_3) 98% ee(*S*)); ^1H NMR (CDCl_3) δ 1.21 (d, 3H, CH_3 , $J = 6.2$ Hz), 1.51-1.65 (m, 3H, CH_2 and OH), 1.77-1.97 (m, 2H, CH_2), 3.56 (t, 2H, CH_2 , $J = 6.4$), 3.79-3.88 (m, 1H, CH); IR (neat) 721, 949, 1080, 1127, 1310, 1377, 1447, 2969, 3326 cm^{-1} ; Anal. Calcd. for $\text{C}_5\text{H}_{11}\text{OCl}$: C, 48.99%; H, 9.04%. Found: C, 49.06%; H, 9.02%.

(S)-1-(2-Thienyl)ethanol ((S)-41b): **41a** (1.75 g, 13.9 mmol) was converted to (S)-**41b** by APG4. Yield 48% (847 mg), ee 99% (Determined by GC analysis. GC conditions: DEX, 120 $^{\circ}\text{C}$, R: 9.3 min, S: 10.1 min); $[\alpha]_{\text{D}}^{23} -22.2$ (c 0.951, C_6H_6)(lit.⁴⁹ $[\alpha]_{\text{D}} -17.8$ (C_6H_6) 72% ee(*S*)); ^1H NMR (CDCl_3) δ 1.58 (d, 3H, CH_3 , $J = 6.6$ Hz), 2.14 (d, 1H, OH, $J = 4.6$ Hz), 5.12 (dq, 1H, CH, $J = 4.8, 6.4$ Hz), 6.93 - 6.99 (m, 2H), 7.21 - 7.25 (m, 1H); ^{13}C NMR (CDCl_3) δ 25.3 (s), 66.3 (s),

123.2 (s), 124.4 (s), 126.6 (s), 149.8 (s); IR (neat) 700, 851, 1070, 1372, 2975, 3355 cm^{-1} ; Anal. Calcd. for $\text{C}_6\text{H}_8\text{OS}$: C, 56.22%; H, 6.29%. Found: C, 56.24%; H, 6.43%.

(-)-1-(3-Thienyl)ethanol ((-)-42b): **42a** (4.51 g, 35.8 mmol) was converted to (*S*)-**42b** by APG4. Yield 45% (2.08 g), ee 99% (Determined by GC analysis. GC conditions: DEX, 120 $^{\circ}\text{C}$, (+): 10.3 min, (-): 11.0 min); $[\alpha]_{\text{D}}^{24}$ -25.1 (*c* 0.993, EtOH); ^1H NMR (CDCl_3) δ 1.48 (d, 3H, CH_3 , J = 6.2 Hz), 2.43 (s, 1H), 4.91 (q, 1H, CH, J = 6.3 Hz), 7.05 - 7.09 (m, 1H), 7.14 - 7.16 (m, 1H), 7.26 - 7.29 (m, 1H); ^{13}C NMR (CDCl_3) δ 24.4 (s), 66.4 (s), 120.1 (s), 125.7 (s), 126.1 (s), 147.3 (s); IR (neat) 786, 850, 1078, 1418, 2974, 3349 cm^{-1} ; Anal. Calcd. for $\text{C}_6\text{H}_8\text{OS}$: C, 56.22%; H, 6.29%. Found: C, 56.29%; H, 6.38%.

(*S*)-2,2,2-Trifluoro-1-phenylethanol ((*S*)-1Fb): **1Fa** (205 mg, 1.18 mmol) was converted to (*S*)-**1Fb** (175 mg) by APG4; GC conditions: DEX 130 $^{\circ}\text{C}$, S: 9.6 min, R: 10.2 min; $[\alpha]_{\text{D}}^{24}$ +30.4 (*c* 1.56, CHCl_3); +25.1 (*c* 0.81, CCl_4)(lit.⁵⁰ $[\alpha]_{\text{D}}^{20}$ -25.1 (*c* 3, CCl_4) >99% ee(*R*)); ^1H NMR (CDCl_3) δ 2.63 (d, 1H, OH, J = 4.6 Hz), 5.02 (dq, 1H, CH, J (d) = 4.5 Hz, J (q) = 6.8 Hz) and 7.37-7.51 (m, 5H, Ph); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -79 (d, J = 6.6 Hz); IR (neat) 633, 706, 760, 835, 866, 1030, 1063, 1128, 1173, 1267, 1358, 1458, 1497, 3407 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_7\text{OF}_3$: C, 54.55; H, 4.01%. Found: C, 54.24%; H, 4.09%.

(*S*)-2,2,2-Trifluoro-1-(*p*-chlorophenyl)ethanol ((*S*)-7Fb): **7Fa** (429 mg, 2.06 mmol) was converted to (*S*)-**7Fb** (350 mg) by APG4; GC conditions: CPCD 140 $^{\circ}\text{C}$, S: 13.7 min, R: 14.5 min; $[\alpha]_{\text{D}}^{24}$ +33.1 (*c* 0.914, EtOH)(lit.⁵¹ $[\alpha]_{\text{D}}^{20}$ -19.0 (*c* 1.05, EtOH) 82.5% ee(*R*)); ^1H NMR (CDCl_3) δ 2.88 (s, 1H, OH), 5.00 (q, 1H, CH, J = 6.5), 7.35 - 7.45 (m, 4H, Ph); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -79 (d, J = 6.4 Hz); IR (KBr) 584, 685, 767, 802, 870, 1015, 1071, 1094, 1128, 1179, 1248, 1352, 1410, 1497, 1601, 3382 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_6\text{OCIF}_3$: C, 45.63; H, 2.87%. Found: C, 45.81%; H, 2.99%.

(*S*)-2,2,2-Trifluoro-1-(*p*-bromophenyl)ethanol ((*S*)-10Fb): **10Fa** (504 mg, 1.99 mmol) was converted to (*S*)-**10Fb** (406 mg) by APG4; GC conditions: CPCD 150 $^{\circ}\text{C}$, S: 13.9 min, R: 14.8 min; $[\alpha]_{\text{D}}^{24}$ +30.25 (*c* 0.862, EtOH)(lit.⁵¹ $[\alpha]_{\text{D}}^{20}$ -21.5 (*c* 1.04, EtOH) 82.2% ee(*R*)); ^1H NMR (CDCl_3) δ 2.73 (s, 1H, OH), 4.99 (q, 1H, CH, J = 6.5), 7.33 - 7.37 (m, 2H, Ph), 7.51 - 7.58 (m, 2H, Ph); ^{19}F NMR ($\text{CDCl}_3\text{-CFCl}_3$) δ -79 (d, J = 6.2 Hz); IR (KBr) 503, 584, 673, 729, 801, 868, 1011, 1076, 1127, 1181, 1248, 1360, 1404, 1493, 1595, 3366 cm^{-1} ; Anal. Calcd. for $\text{C}_8\text{H}_6\text{OBrF}_3$:

C, 37.68%; H, 2.37%. Found: C, 37.68%; H, 2.32%.

(S)-1,1,1-Trifluoro-3-phenyl-2-propanol ((S)-21Fb): **21Fa** (412 mg, 2.19 mmol) was converted to **(S)-21Fb** (307 mg) by APG4; GC conditions: CPCD 140 °C, R: 6.5 min, S: 7.0 min; $[\alpha]^{25}_{\text{D}} -45.5$ (*c* 0.95, CHCl₃)(lit.⁵² $[\alpha]^{23}_{\text{D}} -28.2$ (*c* 0.88, CHCl₃) 98% ee(*S*)); ¹H NMR (CDCl₃) δ 2.15 (d, 1H, OH, *J* = 5.4 Hz), 2.84 (q, 1H, CH₂, *J* = 10.0, 14.2 Hz), 3.06 (q, 1H, CH₂, *J* = 3.0, 14.2 Hz), 4.05 - 4.24 (m, 1H, CH), 7.22 - 7.40 (m, 5H, Ph); ¹⁹F NMR (CDCl₃-CFCl₃) δ -80 (d, *J* = 6.4 Hz); IR (neat) 702, 748, 1096, 1130, 1169, 1275, 1499, 3432 cm⁻¹; Anal. Calcd. for C₉H₉OF₃: C, 56.48%; H, 4.77%. Found: C, 56.55%; H, 4.80%.

(R)-2,2,2-trifluoro-1-(2-thienyl)ethanol ((R)-41b). **41Fa** (8.6 mmol, 1.55 g) was converted to **(R)-41Fb** (1.32 g) by APG4; GC conditions: DEX, 130 °C, R: 11.6 min, S: 12.9 min; $[\alpha]^{25}_{\text{D}} +27.6$ (*c* 0.885, MeOH); ¹H NMR (CDCl₃) δ 2.91 (d, 1H, OH, *J* = 4.8 Hz), 5.21-5.32 (m, 1H, CH), 7.02-7.07 (m, 1H), 7.19-7.21 (m, 1H), 7.37-7.41 (m, 1H); ¹⁹F NMR (CDCl₃-CFCl₃) δ -79.2 (d, *J* = 6.2 Hz); IR (KBr) 3422, 1447, 1354, 1260, 1217, 1182, 1127, 1065, 860, 833, 712 cm⁻¹; Anal. Calcd for C₆H₅OF₃S: C, 39.56%; H, 2.77%. Found: C, 39.33%; H, 2.75%.

(+)-2,2,2-Trifluoro-1-(3-thienyl)ethanol ((+)-42Fb): **42Fa** (984 mg, 5.46 mmol) was converted to **(+)-42Fb** (862 mg) by APG4; GC conditions: DEX 140 °C, (+): 6.4 min, (-): 7.0 min; $[\alpha]^{26}_{\text{D}} +31.6$ (*c* 0.46, MeOH); ¹H NMR (CDCl₃) δ 2.60 (d, 1H, OH, *J* = 4.8), 5.07 - 5.19 (m, 1H, CH), 7.17 - 7.19 (m, 1H), 7.35 - 7.39 (m, 1H), 7.45 - 7.47 (m, 1H) cm⁻¹; ¹³C NMR (CDCl₃) δ 69.4 (q, CH, *J* = 33.1 Hz), 124.1 (q, CF₃, *J* = 281 Hz), 124.8, 126.1, 126.6, 134.9; ¹⁹F NMR (CDCl₃-CFCl₃) δ -79 (d, *J* = 6.2 Hz); IR (neat) 693, 791, 843, 1071, 1127, 1167, 1271, 3395 cm⁻¹; HRMS for (C₆H₅OF₃S)⁺: Calcd: 182.0013, Found: 182.0023.

(R)-2-Fluoro-1-phenylethanol ((R)-43b): **43a** (200 mg, 1.45 mmol) was converted to **(R)-43b** (189 mg) by APG4; GC conditions: DEX 130 °C, S: 8.8 min, R: 9.6 min; $[\alpha]^{24}_{\text{D}} -52.3$ (*c* 1.08, MeOH)(lit.^{35b} $[\alpha]^{23}_{\text{D}} -76.2$ (*c* 3, MeOH) 95.4% ee(*R*)); ¹H NMR (CDCl₃) δ 2.65 (d, 1H, OH, *J* = 2.2 Hz), 4.42 (octet, 1H, CH₂, *J* = 8.1, 9.5, 49 Hz), 4.50 (octet, 1H, CH₂, *J* = 3.4, 9.5, 47 Hz), 5.00 (m, 1H, CH), 7.37 (m, 5H, Ph); ¹³C NMR (CDCl₃) δ 73.0 (d, CH, *J* = 19.8 Hz), 87.2 (d, CH₂F, *J* = 174 Hz), 126.3 (Ph), 128.4 (Ph), 128.6 (Ph), 138.1 (d, Ph, *J* = 8.3); ¹⁹F NMR (CDCl₃ - CFCl₃) δ -221 (dt, *J* = 14.1, 48 Hz); IR (neat) 702, 758, 897, 1011, 1198, 1454, 1495, 3397 cm⁻¹; Anal. Calcd. for C₈H₉OF C, 68.56%; H, 6.47%. Found C, 68.56%; H, 6.64%.

(*R*)-2,2-Difluoro-1-phenylethanol ((*R*)-44b): **44a** (240 mg, 1.54 mmol) was converted to (*R*)-**44b** (240 mg) by APG4; GC conditions: DEX 140 °C, S: 6.7 min, R: 7.2 min; $[\alpha]^{24}_{\text{D}} -11.5$ (*c* 1.00, CH₂Cl₂)(lit.^{35b} $[\alpha]^{23}_{\text{D}} -14.27$ (*c* 3, CH₂Cl₂) 84.66% ee(*R*)); ¹H NMR (CDCl₃) δ 2.62 (d, 1H, OH, *J* = 3.2 Hz), 4.81 (m, 1H, CH), 5.76 (dt, 1H, CHF₂, *J* (d) = 4.8 Hz, *J* (t) = 56 Hz), 7.41 (m, 5H, Ph); ¹³C NMR (CDCl₃) δ 73.6 (t, CHOH, *J* = 24.5 Hz), 115.8 (t, CHF₂, *J* = 245 Hz), 127.1 (Ph), 128.7 (Ph), 129.0 (Ph), 135.8 (t, Ph, *J* = 3.4 Hz); ¹⁹F NMR (CDCl₃ - CFCl₃) δ -128.1 (dd, *J* = 4.2, 56 Hz), -128.0 (dd, *J* = 3.7, 56); IR (neat) 700, 762, 1071, 1204, 1456, 1497, 3410 cm⁻¹; Anal. Calcd. for C₈H₈OF₂: C, 60.76; H, 5.10%. Found: C, 60.67%; H, 5.27%.

(*R*)-2,2-Dichloro-1-phenylethanol ((*R*)-45b): **45a** (461 mg, 2.44 mmol) was converted to (*R*)-**45b** (63.1 mg) by APG4; GC conditions: CPCD 150 °C, S: 19.8 min, R: 20.9 min; $[\alpha]^{21}_{\text{D}} -11.8$ (*c* 1.26, CH₂Cl₂)(lit.⁵³ $[\alpha]^{22}_{\text{D}} -15.0$ (*c* 3, CH₂Cl₂) 45.7% ee(*R*)); ¹H NMR (CDCl₃) δ 2.87 (d, 1H, OH, *J* = 4.0 Hz), 4.98 (dd, 1H, CH, *J* = 3.8, 5.4 Hz), 5.82 (d, 1H, CHCl₂, *J* = 5.4 Hz), 7.36-7.44 (m, 5H, Ph); IR (KBr) 501, 600, 637, 696, 731, 770, 855, 951, 1026, 1069, 1101, 1190, 1229, 1292, 1335, 1420, 1451, 1497, 3362 cm⁻¹; Anal. Calcd. for C₈H₈OCl₂: C, 50.29; H, 4.22%. Found: C, 50.26%; H, 4.23%.

(*S*)-2-Chloro-2,2-difluoro-1-phenylethanol ((*S*)-47b): **47a** (390 mg, 2.05 mmol) was converted to (*S*)-**47b** (322 mg) by APG4; GC conditions: G-TA 125 °C, R: 11.4 min, S: 11.7 min; $[\alpha]^{25}_{\text{D}} +22.2$ (*c* 1.90, CHCl₃)(lit.⁵⁴ $[\alpha]^{21}_{\text{D}} -13.82$ (*c* 1.01, CHCl₃) 73% ee(*R*)); ¹H NMR (CDCl₃) δ 2.91 (d, 1H, OH, *J* = 4.6), 5.06 (dq, 1H, CH, *J* = 4.4, 7.5), 7.39 - 7.50 (m, 5H, Ph); ¹⁹F NMR (CDCl₃-CFCl₃) δ -65 (dd, *J* = 8.8, 165 Hz), -63 (dd, *J* = 7.2, 165 Hz); IR (neat) 658, 698, 739, 814, 851, 978, 1020, 1067, 1117, 1196, 1456, 1497, 3420 cm⁻¹; Anal. Calcd. for C₈H₇OClF₂: C, 49.90%; H, 3.66%. Found: C, 49.60%; H, 3.68%.

(*R*)-1,1,1-Trifluoro-3-phenylthioprop-2-ol ((*R*)-48b). **48a** (100 mg, 0.45 mmol) was converted to (*R*)-**48b** (91 mg) by APG4; GC conditions: CPCD 150 °C; $[\alpha]^{25}_{\text{D}} -79.0$ (*c* 0.428, MeOH), lit.⁵⁵ $[\alpha]^{23}_{\text{D}} -77.7$ (*c* 0.38, MeOH, >99% ee (*R*)); ¹H NMR (CDCl₃) δ 2.86 (d, 1H, OH, *J* = 4.4 Hz), 3.01 (dd, 1H, SCH₂, *J* = 14.2, 10.0), 3.32 (dd, 1H, SCH₂, *J* = 14.2, 2.9 Hz), 3.98 (m, 1H, CH), 7.25-7.45 (m, 5H, Ph); ¹⁹F NMR (CDCl₃-CFCl₃) δ -79.3 (d, *J* = 6.4 Hz); IR (neat) 3416, 1584, 1481, 1441, 1273, 1169, 1128, 866, 741 cm⁻¹; Anal. Calcd. for C₉H₉OF₃S: C, 48.64%; H, 4.08%. Found: C, 48.71%; H, 4.06%.

(S)-1,1,1-Trifluoro-5-phenylthiopentane-2-ol ((S)-49b). **49a** (100 mg, 0.40 mmol) was converted to **(R)-49b** (79 mg) by APG4; HPLC conditions (OD, eluent, n-Hex / 2-propanol = 4 / 1, 0.5 mL/min); $[\alpha]_D^{25}$ -18.7 (c 0.705, CHCl₃); ¹H NMR (CDCl₃) δ 1.68-1.94 (m, 4H, CH₂), 2.12 (d, 1H, OH, *J* = 5.8 Hz), 2.91-3.00 (m, 2H, CH₂), 3.87-3.94 (m, 1H, CH), 7.14-7.37 (m, 5H, Ph); ¹⁹F NMR (CDCl₃-CFCl₃) δ -80.6 (d, *J* = 6.8 Hz); IR (neat) 3409, 1584, 1481, 1439, 1279, 1173, 1127, 1026, 741, 693 cm⁻¹; Anal. Calcd for C₁₁H₁₃OF₃S: C, 52.79%; H, 5.24%. Found: C, 52.81%; H, 5.35%.

(R)-1,1,1-trifluoro-3-octylthiopropene-2-ol ((R)-50b). **50a** (153 mg, 0.60 mmol) was converted to **(R)-50b** (54 mg) by APG4; GC conditions: G-TA 105 °C for 100 min followed by 5 °C/min, He: 2 mL/min; $[\alpha]_D^{25}$ -39.9 (c 0.456, CHCl₃), lit.^{18j} $[\alpha]_D^{24}$ -36.5 (c 2.17, CHCl₃, >95% ee(*R*)); ¹H NMR (CDCl₃) δ 0.87 (t, 3H, CH₃, *J* = 6.4 Hz), 1.14 - 1.40 (m, 10H, CH₂), 1.51 - 1.59 (m, 2H, CH₂), 2.55 (t, 2H, SCH₂, *J* = 7.3 Hz), 2.71 (dd, 1H, CH_aH_bCHOH, *J* = 9.4, 14.4 Hz), 2.89 (dd, 1H, CH_aH_bCHOH, *J* = 3.4, 14.4 Hz), 3.0 (d, 1H, OH, *J* = 4.8), 3.95 - 4.10 (m, 1H, CH); ¹⁹F NMR (CDCl₃-CFCl₃) δ -79.5 (d, *J* = 6.2 Hz); IR (neat) 3418, 2928, 2857, 1273, 1169, 1128 cm⁻¹; Anal. Calcd. for C₁₁H₂₁OF₃S: C, 51.14%; H, 8.19%. Found: C, 50.85%; H, 8.43%.

(+)-1,1,1-Trifluoro-3-(1,3-dithian-2-yl)propane-2-ol ((+)-51b). **51a** (103 mg, 0.48 mmol) was converted to **(R)-51b** (44 mg) by APG4; GC condition: CPCD 160 °C; $[\alpha]_D^{24}$ +4.86 (c 0.617, CHCl₃) ¹H NMR (CDCl₃) δ 1.94-2.16 (m, 2H, CH₂), 2.71-3.04 (m, 4H, SCH₂), 3.09 (d, 1H, OH, *J* = 5.8 Hz), 4.16 (d, 1H, CH, *J* = 5.6 Hz), 4.20-4.32 (m, 1H, CHCF₃); ¹⁹F NMR (CDCl₃-CFCl₃) δ -75.8 (d, *J* = 6.0 Hz); IR (neat) 3320, 2915, 1426, 1281, 1263, 1169, 1121 cm⁻¹; Anal. Calcd for C₆H₉OF₃S₂: C, 33.02%; H, 4.16%. Found: C, 32.79%; H, 4.22%.

General Procedure for the Reduction of Ketones by the B-Enzyme in a Preparative Scale. A ketone (0.80 mmol), NADPH (13 mg), the B-enzyme (10 units), D-glucose-6-phosphate disodium salt hydrate (0.88 mmol) and D-glucose-6-phosphate dehydrogenase (25 units) were added to 50 ml of HEPES buffer (0.1 M, pH 7.0). The mixture was stirred at 30 °C for 20 h at 130 rpm, and then extracted with ether. The combined ether solution was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by distillation with a Kugelrohr apparatus.

(S)-2-Fluoro-1-phenylethanol ((S)-43b): **43a** (119 mg, 0.862 mmol) was converted to **(S)-11b** (110 mg); $[\alpha]_D^{23}$ +52.5 (c 0.94, MeOH). The NMR and IR spectra are in accord with those for

(*R*)-**43b**. Found: C, 68.50%; H, 6.57%.

(*S*)-**2,2-Difluoro-1-phenylethanol** ((*S*)-**44b**): **44a** (124 mg, 0.795 mmol) was converted to (*S*)-**44b** (119 mg); $[\alpha]_D^{25} +19.4$ (*c* 0.96, CH₂Cl₂). The NMR and IR spectra are in accord with those for (*R*)-**44b**. Found: C, 60.94%; H, 5.16%.

Determination of Absolute Configurations.

(*S*)-1-Phenylethanol (**1b**) from chiral *m*-bromophenylethanol (**9b**) obtained by APG4 reduction of *m*-Bromoacetophenone (**9a**).

Chiral *m*-bromophenylethanol (**9b**) (137 mg, 0.68 mmol) obtained by APG4 reduction of *m*-bromoacetophenone (**9a**), ethanol (13 ml), sodium hydroxide (400 mg) and 5% palladium on carbon (45 mg) was stirred overnight under an atmosphere of hydrogen at room temperature. The catalyst was removed by filtration, and the filtrate was neutralized with 1 N HCl. Brine was added to the mixture, and the entire mixture was extracted with ether. The combined extracts were washed with water, aqueous sodium bicarbonate and water and dried over Na₂SO₄, and the solvent was evaporated. The residue was purified by silica gel column chromatography (eluent, hexane : ethyl acetate = 4 : 1) followed by distillation with a Kugelrohr apparatus. ¹H NMR spectrum of the product was identical with that of 1-phenylethanol. Ee of the product was determined to be 88% by GC analysis (GC conditions: CPCD, 110 °C). Yield: 64% (53 mg); $[\alpha]_D^{25} -46.8$ (*c* 1.17, CHCl₃) (lit.³³ $[\alpha]_D^{25} -57$ (*c* 5.12, CHCl₃) *S*).

(*S*)-2-Undecanol from (*S*)-propylene oxide.

CuI (0.2 mmol, 40 mg) and Me₂S (0.2 mL) were added to a cold (-20 °C) solution of *n*-octylmagnesium bromide (0.93 M in THF, 2.4 mL) under an atmosphere of argon. Then, a THF (2 mL) solution of (*S*)-propene oxide (116 mg, 2 mmol) was added dropwise to the mixture, and the mixture was stirred for 2 h at the same temperature. The reaction was quenched by aqueous NH₄Cl, the mixture was acidified with 1N HCl, and the product was extracted with ether. The combined ether extracts were washed with water, aqueous sodium bicarbonate, and water, and dried over anhydrous Na₂SO₄, and the solvent was evaporated. The crude product was purified by silica gel column chromatography (eluent, hexane : ethyl acetate = 8 : 1 to 5 : 1) followed by distillation with a Kugelrohr apparatus. NMR spectrum of the product was identical with that of 2-undecanol. Yield: 70% (240 mg); $[\alpha]_D^{25} +7.44$ (*c* 1.27, EtOH). The product was acetylated with acetylchloride and pyridine in CH₂Cl₂ to determine ee by GC analysis (GC conditions: CPCD, 120 °C); ee 99%.

VII. References

- 1 (a) Seebach, D.; Imwinkelried, R.; Weber, T. In *Modern Synthetic Methods 1986*; Scheffold, R., Ed.; Springer-Verlag; Berlin, **1986**; Vol. 4, pp 125-259. (b) Helmchen, G.; Karge, R.; Weetman, J. In *Modern Synthetic Methods 1986*; Scheffold, R., Ed.; Springer-Verlag; Berlin, **1986**; Vol. 4, pp 261-306. (c) Brown, H. C.; Jadhav, P. K.; Singram, B. In *Modern Synthetic Methods 1986*; Scheffold, R., Ed.; Springer-Verlag; Berlin, **1986**; Vol. 4, pp 307-356. (d) Santaniello, E., Ferraboschi, P., Grisenti, P., Manzocchi, A. *Chem. Rev.* **1992**, 92 1071-1140.
- 2 (a) Lewis, E. S.; Boozer, C. E. *J. Am. Chem. Soc.* **1952**, 74, 308. (b) Ward, A. M. *Org. Synth., Coll. Vol.* **1943**, 2, 159. (c) Shoppee, C. W.; Coll, J. C. *J. Chem. Soc. (C)*, **1970**, 1124. (d) Carman, R. M.; Shaw, I. M. *Aust. J. Chem.* **1976**, 29, 133.
- 3 (a) Degerbeck, F.; Fransson, B.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1*, **1992**, 245. (b) Corey, E. J.; Wei-guo, S. *Tetrahedron Lett.* **1990**, 31, 3833.
- 4 Fabiano, E.; Golding, B. T.; Sadeghi, M. M. *Synthesis*, **1987**, 190.
- 5 (a) Leroy, J.; Hebert, E.; Wakselman, C. *J. Org. Chem.* **1979**, 44, 3406. (b) Hamman, S.; Barrelle, M.; Tetaz, F.; Beguin, C. G. *J. Fluorine Chem.* **1987**, 37, 85. (c) Kopecky, J.; Smejkal, J. *Collect. Czech. Chem. Commun.* **1980**, 45, 2971.
- 6 (a) Servi, S. *Microbial Reagents in Organic Synthesis*, NATO ASI Series C, Kluwer, **1992**, Dordrecht. (b) Faber, K. *Biotransformations in Organic Chemistry*, 2nd Ed. Springer, **1995**, Berlin. (c) Roberts, S. M.; Turner, N. J.; Willetts, A. J.; Turner, M. K. *Introduction to Biocatalysis Using Enzymes and Micro-organisms*, Cambridge University Press, **1995**, New York. (d) Azerad, R. *Bull Soc Chim Fr*, **1995**, 132, 17. (e) Roberts, S. M.; Turner, N. J. *J. Biotechnology*, **1992**, 22, 227. (f) Turner, N. J. *Nat. Prod. Rep.* **1994**, 11, 1. (g) Sih, C. J.; Chen, C.-S. *Angew. Chem., Int. Ed. Engl.*, **1984**, 23, 570. (h) Pratt, A. J. *Chemistry in Britain*, **1989**, 282. (i) Klivanov, A. *Acc. Chem. Res.* **1990**, 23, 114. (j) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1*, **1998**, 157. (k) Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1*, **1999**, 1. (l) McCoy, M. *Chem. Eng. News*, January 4, **1999**, 10. (m) Pereira, R. d. S. *Crit. Rev. Biotech.* **1998**, 18, 25, and references sited therein.
- 7 (a) MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. *Biochemistry*, **1964**, 3, 838. (b) Servi, S. *Synthesis*, **1990**, 1.

- 8 (a) Davies, J.; Jones, J. B. *J. Am. Chem. Soc.* **1979**, *101*, 5405. (b) Haslegrave, J. A.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, *104*, 4666. (c) Jones, J. B.; Beck, J. F. *Applications of Biochemical Systems in Organic Chemistry*. Jones, J. B.; Sih, C. J.; Perlman, D. Ed. John Wiley & Sons, Inc. **1976**, *Part I*, New York, pp 107-401.
- 9 (a) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162. (b) Keinan, E.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 3474.
- 10 Zheng, C.; Pham, V. T.; Phillips, R. *Catalysis Today* **1994**, *22*, 607.
- 11 (a) Hummel, W. *Appl. Microbiol. Biotechnol.* **1990**, *34*, 15. (b) Bradshaw, C. W.; Hummel, W.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1532.
- 12 (a) Shen, G.-J.; Wang, Y.-F.; Bradshaw, C.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1990**, 677. (b) Bradshaw, C. W.; Fu, H.; Shen, G.-J.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 1526.
- 13 Adlercreutz, P. *Enzyme Microb. Technol.* **1991**, *13*, 9.
- 14 Bortolini, O.; Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Guerrini, A.; Medici, A. *J. Org. Chem.* **1997**, *62*, 1854.
- 15 (a) Azerad, R.; Buisson, D. *Microbial Reagents in Organic Synthesis*, NATO ASI Series C; Servi, S. Ed. Kluwer Academic Publishers, **1992**, Dordrecht; pp 421-440. (b) Nakamura, K.; Yoneda, T.; Miyai, T.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1988**, *29*, 2453. (c) Nakamura, K.; Takano, S.; Terada, K.; Ohno, A. *Chem. Lett.* **1992**, 951. (d) Nakamura, K.; Takano, S.; Ohno, A. *Tetrahedron Lett.* **1993**, *34*, 6087. (e) Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett.* **1995**, *36*, 265.
- 16 (a) Nakamura, K. *Microbial Reagents in Organic Synthesis*, NATO ASI Series C; Servi, S. Ed. Kluwer Academic Publishers, **1992**, Dordrecht; pp 389-398. (b) Zhou, B.-n; Gopalan, A. S.; VanMiddlesworth, F.; Shieh, W.-R; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925. (c) Nakamura, K.; Kondo, S.; Nakajima, N.; Ohno, A. *Tetrahedron*, **1995**, *51*, 687. (d) Nakamura, K.; Miyai, T.; Fukushima, K.; Kawai, Y.; Babu, B. R.; Ohno, A. *Bull. Chem. Soc. Jpn.*, **1990**, *63*, 1713. (e) Nakamura, K.; Kawai, Y.; Miyai, T.; Ohno, A. *Tetrahedron Lett.* **1990**, *31*, 3631. (f) Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem.* **1991**, *56*, 4778. (g) Nakamura, K.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 2927. (h) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Tetrahedron : Asymm.* **1993**, *4*, 1253. (i) Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2738. (j)

- Nakamura, K.; Takano, S; Ohno, A. *Tetrahedron Lett.* **1993** 34, 6067. (k) Nakamura, K.; Kondo, S.; Ohno, A. *Bioorg. Med. Chem.* **1994**, 2, 433. (l) Fujisawa, T.; Itoh, T.; Sata, T. *Tetrahedron Lett.* **1984** 25, 5083.
- 17 Goodhue, C. T.; Rosazza, J. P.; Peruzzotti, G. P. *Manual of Industrial Microbiology and Biotechnology*. Demain, A. L.; Solomon, N. A. Eds.; American Society for Microbiology, **1986**, Washington D.C. pp 97-121.
- 18 (a) Kitazume, T.; Yamazaki, T. *Experimental Methods in Organic Fluorine Chemistry*, Kodansha, **1998**, Tokyo. (b) Nohira, H. *J. Synth. Org. Chem. Jpn.* **1991**, 49, 467. (c) Tanaka, Y.; DeLuca, H. F.; Kobayashi, Y.; Ikekawa, N. *Arch. Biochem. Biophys.* **1984**, 229, 348. (d) Welch, J. T. *Tetrahedron*, **1987**, 43, 3123. (e) Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, 91, 49. (f) Lin, J. T.; Yamazaki, T.; Kitazume, T. *J. Org. Chem.* **1987**, 52, 3211. (g) Kitazume, T.; Kobayashi, T.; Yamamoto, T.; Yamazaki, T. *J. Org. Chem.* **1987**, 52, 3218. (h) Kitazume, T.; Nakayama, Y. *J. Org. Chem.* **1986**, 51, 2795. (i) Sakai, T.; Yan, F.; Kashino, S.; Uneyama, K. *Tetrahedron*, **1996**, 52, 233. (j) Petschen, I.; Malo, E. A.; Bosch, M. P.; Guerrero, A. *Tetrahedron: Asymm.* **1996**, 7, 2135. (k) Kitazume, T.; Yamazaki, T. In *ACS Symposium Series No. 456, Selective Fluorination in Organic and Bioorganic Chemistry*; Welch, J. T., Ed.; American Chemical Society, 1991; pp 175-185. (l) Resnati, G. *Tetrahedron*, **1993**, 49, 9385.
- 19 Sugai, T.; Katoh, O.; Ohta, H. *Tetrahedron*, **1995**, 51, 11987.
- 20 Hirschbein, B. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1982**, 104, 4458.
- 21 (a) Vandecasteele, J.-P.; Lemal, J. *Bull. Soc. Chim. Fr.* **1980**, 101. (b) Wong, C.-H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, 107, 4028.
- 22 (a) Tischer, W.; Tiemeyer, W.; Simon, H. *Biochimie*, **1980**, 62, 331. (b) Wichmann, R.; Wandrey, C.; Bückmann, A. F.; Kula, M.-R. *Biotechnol. Bioeng.* **1981**, 23, 2789.
- 23 Jakovac, I. J.; Goodbrand, H. B.; Lok, K. P.; Jones, J. B. *J. Am. Chem. Soc.* **1982**, 104, 4659.
- 24 Nakamura, K.; Inoue, Y.; Ohno, A. *Tetrahedron Lett.* **1994**, 35, 4375.
- 25 Brown, H. C.; Ichikawa, K. *Tetrahedron*, **1957**, 1, 221.
- 26 Nakamura, K.; Matsuda, T.; Ohno, A. *Tetrahedron Asymmetry*, **1996**, 7, 3021.
- 27 Nakamura, K.; Inoue, Y.; Matsuda, T.; Ohno, A. *Tetrahedron Lett.* **1995**, 36, 6263.
- 28 (a) Calet, S.; Urso, F.; Alper, H. *J. Am. Chem. Soc.* **1989**, 111, 931. (b) Brown, H. C.; Pai,

- G. G. *J. Org. Chem.* **1983**, *48*, 1784. (c) Lucchi, O. D.; Buso, M.; Modena, G. *Tetrahedron Lett.* **1987**, *28*, 107. (d) Atkins, R. K.; Frazier, J.; Moore, L. L.; Weigel, L. O. *Tetrahedron Lett.* **1986**, *27*, 2451. (e) Niwa, M.; Jiang, P.-F.; Hirata, Y. *Chem. Pharm. Bull.* **1987**, *35*, 108.
- 29 (a) Midland, M. M.; Kazubski, A. *J. Org. Chem.* **1982**, *47*, 2495. (b) Noyori, R.; Tomino, I.; Tanimoto, Y.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6709. (c) de Vries, E. F.-J.; Brussee, J.; Kruse, C. G.; van der Gen, A. *Tetrahedron Asymmetry*, **1994**, *5*, 377. (d) Brown, H. C.; Chandrasekharan, J.; Ramachandran, P. V. *J. Am. Chem. Soc.* **1988**, *110*, 1539. (e) Carter, M. B.; Schiøtt, B.; Gutiérrez, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 11667. (f) Ohkuma, T.; Ooka, H.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1995**, *117*, 10417. (g) Püntener, K.; Schwink, L.; Knochel, P. *Tetrahedron Lett.* **1996**, *37*, 8165.
- 30 Weissaman, S. A.; Ramachandran, P. V. *Tetrahedron Lett.* **1996**, *37*, 3791.
- 31 Byrne, K. J.; Swigar, A. A.; Silversteine, R. M.; Borden, J. H.; Stokkink, E. *J. Insect Physiol.* **1974**, *20*, 1895.
- 32 (a) Maurer, B.; Grieder, A.; Thommen, W. *Helv. Chim. Acta*, **1979**, *62*, 44. (b) Maurer, B.; Thommen, W. *Helv. Chim. Acta*, **1979**, *62*, 1096. (c) van Dorp, D. A.; Ward, J. P. *Experientia* **1981**, *37*, 917.
- 33 Kasai, M.; Froussios, C.; Ziffer, H. *J. Org. Chem.* **1983**, *48*, 459.
- 34 (a) Kitazume, T.; Yamazaki, T. *Selective Fluorination in Organic and Bioorganic Chemistry*, ACS Symposium Series No. 456, Welch, J. T. Ed.; American Chemical Society, 1991; pp 175-185 and references cited therein. (b) Kitazume, T.; Nakayama, Y. *J. Org. Chem.* **1986**, *51*, 2795. (c) Xiao, L.; Kitazume, T. *J. Fluorine Chem.* **1997**, *86*, 99. (d) Kitazume, T.; Lin, J. T.; Yamazaki, T. *Tetrahedron: Asymm.* **1991**, *2*, 235. (e) Lin, J. T.; Yamazaki, T.; Kitazume, T. *J. Org. Chem.* **1987**, *52*, 3211. (f) Arnone, A.; Bernardi, R.; Blasco, F.; Cardillo, R.; Resnati, G.; Gerus, I. I.; Kukhar, V. P. *Tetrahedron*. **1998**, *54*, 2809. (g) Ohtsuka, Y.; Katoh, O.; Sugai, T.; Ohta, H. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 483. (h) Petschen, I.; Malo, E. A.; Bosch, M. P.; Guerrero, A. *Tetrahedron: Asymm.* **1996**, *7*, 2135. (i) Kato, K.; Katayama, M.; Gautam, R. K.; Fujii, S.; Kimoto, H. *Biosci. Biotech. Biochem.* **1995**, *59*, 271. (j) Kato, K.; Katayama, M.; Gautam, R. K.; Fujii, S.; Kimoto, H., *J. Ferment. Bioeng.* **1993**, *76*, 178. (k) Carvalho, M.; Okamoto, M. T.; Moran, P. J. S.;

- Rodrigues, J. A. R; *Tetrahedron*, **1991**, 47, 2073. (l) Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *Synthesis*, **1983**, 897. (m) Bucciarelli, M.; Forni, A.; Moretti, I.; Torre, G. *J. C.S. Chem. Comm.* **1978**, 456. (n) Fujisawa, T.; Onogawa, Y.; Sato, A.; Mitsuya, T.; Shimizu, M. *Tetrahedron*, **1998**, 54, 4267. (o) Hamada, H.; Shiromoto, M.; Funahashi, M.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **1996**, 61, 2332.
- 35 (a) Ramachandran, P. V.; Teodorovic, A. V.; Brown, H. C. *Tetrahedron*, **1993**, 49, 1725. (b) Ramachandran, P. V.; Teodorovic, A. V.; Gong, B.; Brown, H. C. *Tetrahedron: Asymm.* **1994**, 5, 1075. (c) Corey, E. J.; Link J. O.; Sarshar, S.; Shao, Y. *Tetrahedron Lett.* **1992**, 33, 7103. (d) Corey, E. J.; Link J. O.; Bakshi, R. K. *Tetrahedron Lett.* **1992**, 33, 7107. (e) Corey, E. J.; Bakshi, R. K. *Tetrahedron Lett.* **1990**, 31, 611. (f) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C.-P.; Singh. V. K. *J. Am. Chem. Soc.* **1987**, 109, 7925. (g) Carrière, A.; Virgili, A.; Figueredo, M. *Tetrahedron: Asymm.* **1996**, 7, 2793. (h) Noyori, R.; Tomino, I.; Tanimoto, Y.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, 106, 6709. (i) Chong, J. M.; Mar, E. K. *J. Org. Chem.* **1991**, 56, 893. (j) Corey, E. J.; Bakshi, R. K.; Shibata, S. *J. Am. Chem. Soc.* **1987**, 109, 5551. (k) Brown, H. C.; Cho, B. T.; Park, W. S. *J. Org. Chem.* **1988**, 53, 1231. (l) Midland, M. M.; McLoughlin, J. I.; Gabriel, J. *J. Org. Chem.* **1989**, 54, 159. (m) Brown, H. C.; Pai, G. G. *J. Org. Chem.* **1985**, 50, 1384. (n) Hawkins. J. M.; Sharpless, K. B. *J. Org. Chem.* **1984**, 49, 3861. (o) Midland, M. M.; Kazubski, A. *J. Org. Chem.* **1982**, 47, 2495. (p) Yamaguchi, S.; Mosher, H. S. *J. Org. Chem.* **1973**, 38, 1870. (q) Nasipuri, D.; Bhattacharya, P. K. *J. Chem. Soc. Perkin Trans I*, **1977**, 576.
- 36 Bott, G.; Field, L. D.; Sternhell, S. *J. Am. Chem. Soc.* **1980**, 102, 5618.
- 37 (a) Chen, T.-A.; Wu, X.; Rieke, R. D. *J. Am. Chem. Soc.* **1995**, 117, 233. (b) Engelmann, G.; Kossmehl, G.; Heinze, J.; Tschuncky, P.; Jugelt, W.; Welzel, H.-P. *J. Chem. Soc., Perkin Trans. 2*, **1998**, 169. (c) Langeveld-Voss, B. M. W.; Janssen, R. A. J.; Christiaans, M. P. T.; Meskers, S. C. J.; Dekkers, H. P. J. M.; Meijer, E. W. *J. Am. Chem. Soc.* **1996**, 118, 4908. (d) Blettner, C. G.; König, W. A.; Stenzel, W.; Schotten T. *J. Org. Chem.* **1999**, 64, 3885. (e) McCullough, R. D.; Ewbank, P. C.; Loewe, R. S. *J. Am. Chem. Soc.* **1997**, 119, 633. (f) Faïd. K.; Leclerc. M. *J. Am. Chem. Soc.* **1998**, 120, 5274.
- 38 McConnell, E. E.; Moore, J. A.; Haseman, J. K.; Harris, M. W. *Toxicol. Appl. Pharmacol.* **1978**, 44, 335.

- 39 Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y., *Tetrahedron: Asymmetry*, **1996**, 7, 3285.
- 40 Hayashi, T.; Matsumoto, Y.; Ito, Y. *Tetrahedron: Asymmetry*, **1991**, 2, 601.
- 41 Nakamura, K.; Kawasaki, M.; Ohno, A., *Bull. Chem. Soc. Jpn*, **1996**, 69, 1079.
- 42 Chen, C.-P.; Prasad, K.; Repic, O., *Tetrahedron Lett.* **1991**, 32, 7175.
- 43 Fukumasa, M.; Furuhashi, K.; Umezawa, J.; Takahashi, O.; Hirai, T. *Tetrahedron Lett.* **1991**, 32, 1059.
- 44 Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; Santaniello, E. *Tetrahedron*, **1994**, 50, 10539.
- 45 Imuta, M.; Kawai, K.; Ziffer, H., *J. Org. Chem.* **1980**, 45, 3352.
- 46 Seebach, D.; Giovannini, F.; Lamatsch, B., *Helvetica Chimica Acta*, **1985**, 68, 958.
- 47 Tacke, R.; Linoh, H.; Stumpf, B.; Abraham, W.-R.; Kieslich, K.; Ernst, L., *Z. Naturforsch.* **1983**, 38b, 616.
- 48 Sonnet, P. E. *J. Org. Chem.* **1987**, 52, 3477.
- 49 Meyers, A. I.; Brown, J. D. *Tetrahedron Lett.* **1988**, 29, 5617.
- 50 Holzwarth, G.; Hsu, E. C.; Mosher, H. S.; Faulkner, T. R.; Moscovitz, A. *J. Am. Chem. Soc.* **1974**, 96, 251.
- 51 Ohno, A.; Nakai, J.; Nakamura, K.; Goto, T.; Oka, S. *Bull. Chem. Soc. Jpn.* **1981**, 54, 3486.
- 52 Shimizu, M.; Sugiyama, K.; Fujisawa, T. *Bull. Chem. Soc. Jpn.* **1996**, 69, 2655.
- 53 Weidmann, R.; Schoofs, A. R.; Horeau, A. *Comptes Rendus Acad. Sc. Ser. II*, **1982**, 294, 319.
- 54 Kitazume, T.; Asai, M.; Tsukamoto, T.; Yamazaki, T. *J. Fluorine Chem.* **1992**, 56, 271.
- 55 Shimizu, M.; Sugiyama, K.; Fujisawa, T. *Bull. Chem. Soc. Jpn.* **1996**, 69, 2655.

List of Publications

1. Asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum*
Kaoru Nakamura, Tomoko Matsuda
J. Org. Chem. 1998, **63**, 8957.
2. The Presence of Two Classes of Enzymes of Opposite Stereochemistry in an Organism: One for Fluorinated and Another for Non-fluorinated Substrates
Tomoko Matsuda, Tadao Harada, Nobuyoshi Nakajima, Toshiyuki Itoh, Kaoru Nakamura
J. Org. Chem. Accepted.
3. Microbial deracemization of 1-arylethanol
Kaoru Nakamura, Yuko Inoue, Tomoko Matsuda, Atsuyoshi Ohno
Tetrahedron Lett. 1995, **36**, 6263.
4. Asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum*
Kaoru Nakamura, Kazutada Kitano, Tomoko Matsuda, Atsuyoshi Ohno
Tetrahedron Lett. 1996, **37**, 1629.
5. Different stereochemistry for the reduction of trifluoromethyl ketones and methyl ketones catalyzed by alcohol dehydrogenase from *Geotrichum*
Kaoru Nakamura, Tomoko Matsuda, Toshiyuki Itoh, Atsuyoshi Ohno
Tetrahedron Lett. 1996, **37**, 5727.
6. Asymmetric synthesis of (*S*)-arylalkanols by microbial reduction
Kaoru Nakamura, Tomoko Matsuda, Atsuyoshi Ohno
Tetrahedron: Asymmetry, 1996, **7**, 3021.
7. Asymmetric reduction of trifluoromethyl ketones containing a sulfur functionality by the alcohol dehydrogenase from *Geotrichum*
Kaoru Nakamura, Tomoko Matsuda, Makoto Shimizu, Tamotsu Fujisawa
Tetrahedron, 1998, **54**, 8393.
8. Stereoselective oxidation and reduction by immobilized *Geotrichum candidum* in an organic solvent
Kaoru Nakamura, Yuko Inoue, Tomoko Matsuda, Ibuki Misawa
Perkin Transactions I, 1999, 2397.

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